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BIOLOGICAL IMPACTS AND RECOVERY FROM MARINE DISPOSAL OF METAL MINING WASTE

A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

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DOCTOR OF PHILOSOPHY

By

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MARINE DISPOSAL OF METAL MINING WASTE

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Abstract

Waste from coastal, metal mining operations may be disposed of in the ocean. Studies were conducted using tailings and wastewater (effluent) from a proposed gold mine that is located near Juneau, Alaska, USA. The ability of invertebrates to colonize tailings after obliteration by submarine tailings disposal (STD) was assessed through a field experiment. Trays of tailings and reference sediment were placed on the sea floor and retrieved over a 22 month period. The taxonomic composition, abundance, and biomass of invertebrates that colonized tailings and reference sediment were similar. Therefore, recolonization of invertebrates after obliteration by STD should not be inhibited by the presence of these tailings as a bottom substrate. In a laboratory study, the toxicity of effluent from the milling process was compared for early life stage fish and crustaceans. Common reference species and species that are indigenous to southern Alaska were exposed to effluent. The relationship between effluent concentration and organism response was established for immobilization, paralysis, and death. For each response, the sensitivity of the reference species bracketed that of the indigenous species. An overall ranking of species sensitivity could not be made because it depended on the response that was compared. The source of effluent toxicity was determined for one of the reference species, a crustacean. A simulated effluent was created to duplicate the ionic composition of the actual effluent. Toxicity was compared in effluent, effluent with increased salinity, simulated effluent, and

solutions with adjusted concentrations of ions. Calcium was in excess in the effluent, relative to seawater, and was isolated as the source of toxicity. Sodium deficiency in the effluent, relative to seawater, reduced calcium toxicity.

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Chapter 1

General Introduction

Hard rock mining and milling produce three main types of waste: waste rock; tailings; wastewater. Waste rock is the overburden that is removed from a mine to access ore. Relative to the volume of ore, the volume of waste rock from an open pit mine greatly exceeds that from an underground mine. Disposal of waste rock can alter habitat types and can result in release of dissolved metals to the environment (Johansen *et al.* 1991, Ellis *et al.* 1995). Environmental impacts of waste rock disposal were not addressed in this thesis.

Ore is commonly milled near the site of mining. During milling, ore is crushed and ground to expose metal bearing particles. Froth flotation is the predominate method that is used to separate the metal bearing particles (mineral concentrate) from the non-metal portion (tailings) of ground ore. For precious metals, such as gold, mineral concentrate from froth flotation may be processed at the same site or it may be shipped off-site for further processing. Cyanide leaching is commonly used to extract precious metals from mineral concentrate.

Most of the crushed ore is disposed of as tailings after froth flotation. A portion of the tailings from an underground mine may be placed back underground in spent shafts. The remainder is disposed of on land or underwater. Tailings disposal poses similar risks to the environment as waste rock disposal with regard to habitat alteration

and possible release of metals (Besser and Rabeni 1987, Kelley and Tuovinen 1988, Johansen *et al.* 1991). A portion of this thesis addressed disposal of tailings from froth flotation through a method called submarine tailings disposal (STD).

STD is not an option for most mines and is prohibited in the U.S. The primary potential for STD in the U.S. is in Alaska due to abundant coastal mineral reserves combined with other required, coastal features (Coldwell and Gensler 1993). Where STD is employed, marine impacts are exchanged for terrestrial impacts. As a result, value judgements are required when comparing the environmental risks of STD and terrestrial disposal. Tailings that are disposed of on land are most susceptible to chemical reactivity and erosion (Besser and Rabeni 1987, Kelley and Tuovinen 1988).

Any water that is discharged to the environment after being altered by activities related to mining and milling is wastewater, or effluent. Sources of wastewater include water that has been in contact with a mine pit, underground workings, or tailings, sewage, natural waters that mix with wastewater, and water that is used for ore processing (e.g. U.S. Forest Service 1991). Froth flotation and cyanide leaching require use of water. Water from these processes may be recirculated and re-used at some sites or process water may be discharged to the environment. Some form of water treatment, such as cyanide destruction (McGill and Comba 1990), is commonly employed before discharge. Additional reductions in the concentrations of cyanide and other wastewater components may be accomplished by

retention in a holding pond. A portion of this thesis addressed toxicity of process water from froth flotation and cyanide leaching.

Process water from froth flotation may be used to carry tailings to the point of discharge (Ellis *et al.* 1995). Process water could be removed from tailings prior to discharge through STD, however, residual quantities will remain. Based on an interpretation of the Clean Water Act by the U.S. Environmental Protection Agency, STD is prohibited because the discharge of process waters from froth flotation to navigable waters is prohibited (Baer *et al.* 1992).

The purpose of this thesis was to improve our understanding of biological impacts that result from marine discharge of waste from metal mining and milling operations. Ecological recovery during and after STD and toxicity of process water were addressed. These topics were selected for study based on identification of the greatest knowledge gaps pertaining to environmental impacts of coastal metal mines. Methods were developed and tested in a case study under the belief that case studies are an effective means to acquire a general understanding of the environmental impacts of mining.

Literature Cited

Baer, R.L., G.E. Sherman, and P.D. Plumb. 1992. Submarine disposal of mill tailings from on-land sources - an overview and bibliography. U.S. Bureau of Mines, OFR 89-92, 102 pp.

Besser, J.M. and C.F. Rabeni. 1987. Bioavailability and toxicity of metals leached from lead-mine tailings to aquatic invertebrates. *Environ. Toxicol. Chem.* 6:879-890.

Coldwell, J.R. and E.C. Gensler. 1993. Potential for submarine tailings disposal to affect the availability of minerals from United States coastal areas. U.S. Bureau of Mines, OFR 101-93, 49 pp.

Ellis, D.V., T.F. Pedersen, G.W. Poling, C. Pelletier, and I. Horne. 1995. Review of 23 years of STD: Island Copper Mine, Canada. *Mar. Geores. Geotech.* 13:59-99.

Johansen, P., M.M. Hansen, G. Asmund, and P.B. Nielsen. 1991. Marine organisms as indicators of heavy metal pollution - experience from 16 years of monitoring at a lead zinc mine in Greenland. *Chem. Ecol.* 5:35-55.

Kelley, B.C. and O.H. Tuovinen. 1988. Microbial oxidations of minerals in mine tailings. *Chemistry and Biology of Solid Waste: Dredged Material and Mine Tailings*. W. Salomons and U. Forstner, eds., Springer-Verlag, Berlin, Germany, pp. 33-53.

McGill, S.L. and P.G. Comba. 1990. A review of existing cyanide destruction practices. *Proceedings of the Nevada Wildlife/Mining Workshop*. Reno, Nevada, USA, pp. 172-185.

U.S. Department of Agriculture. 1991. *Draft Environmental Impact Statement Kensington Gold Project*. Juneau Ranger District, Juneau, AK, USA.

Chapter 2

Experimental Comparison of Marine Macrofaunal Colonization in Mine Tailings and Natural Sediment

Abstract

Submarine tailings disposal (STD) obliterates or displaces benthos in areas that receive the highest rates of tailings deposition. An experiment was conducted to assess the ability of macrofauna to recolonize the sea floor after obliteration by submarine tailings disposal (STD). Trays of defaunated, natural, marine sediment, serving as a reference, and trays of flotation tailings from a proposed gold mine, were placed at 21 m depth on the sea floor near Juneau, Alaska. Reference sediment trays, tailings trays, and cores of undisturbed, ambient sediment were collected after 9, 17, and 22 month colonization periods. Total abundance of macrofauna ranged from 14 to 24% lower in tailings than in reference sediment. Total biomass, number of taxa, the average size of an individual (biomass/abundance), and taxa richness using rarefaction methodology were nearly equal or greater in tailings than in reference sediment. Overall, numerically dominant taxa, abundance by feeding type, and abundance by sediment association type were similar in reference sediment and tailings. Based on the identity and abundance of macrofauna, reference sediment and tailings assemblages were indistinguishable using correspondence analysis. The ambient

assemblage was distinguishable from the reference and tailings assemblages for most comparisons. As such, the successional stage of the tray assemblages could not be determined based on comparison to the ambient assemblage. Taxonomic overlap between macrofauna at a candidate STD site and macrofauna in the reference sediment and tailings was low. Regardless of the ambient and candidate STD site assemblages, recolonization of macrofauna after STD would not be greatly influenced by the presence of these tailings as a benthic substrate. Rather, recolonization of macrofauna after obliteration by STD would be dictated by natural recruitment processes.

Introduction

A major challenge for metal mining operations is disposing of tailings in a cost effective manner while minimizing environmental impacts. Foremost among environmental concerns is contamination of aquatic ecosystems with metals that may leach from tailings that are deposited on land. Depending on the mineral composition of tailings, submersion may reduce the risk of metal leaching and may not appreciably alter the chemistry of host waters (Pedersen and Losher 1988). Furthermore, if tailings are deposited on the bottom of a lake or the ocean, the risk of erosion and uncontrolled transport of tailings in surface waters that exists from terrestrial disposal is circumvented.

Submarine tailings disposal (STD) is an option for some mining operations. STD involves discharging tailings as a slurry, below the photic zone, along the bottom of a near-shore, soft-bottom marine habitat (Caldwell and Welsh 1982, Hay *et al.* 1983). The tailings slurry may contain process wastewater (Poling 1982). STD is prohibited in the United States, although exemptions have been considered and STD is employed in other countries (Ellis *et al.* 1995b, Hesse and Ellis 1995, Jones and Ellis 1995). The primary potential for STD in the U.S. is in Alaska due to abundant coastal mineral reserves combined with other required, coastal features (Coldwell and Gensler 1993).

Numerous questions pertaining to the physical, chemical, and biological aspects of STD must be answered to be able to predict the

site-specific environmental impacts. Potential negative consequences of STD include bioaccumulation of metals (Johansen *et al.* 1991), toxicity from milling reagents and dissolved metals (Leduc *et al.* 1975), increased photic zone turbidity (Ellis *et al.* 1995a), and habitat alteration (Poling *et al.* 1993). These risks may be negligible if: 1) tailings are chemically stable in the receiving environment; 2) toxicity of the liquid portion of the tailings slurry is low; 3) tailings are discharged at depth and settle rapidly; 4) the receiving environment is already depositional (Poling 1982, Ellis *et al.* 1994). However, an unavoidable impact of STD is burial and displacement of benthic organisms (e.g. Kathman *et al.* 1983). The rate of benthic recovery after STD is a function of the natural recruitment potential of an ecosystem and the ability of benthos to inhabit tailings.

Most members of the marine benthos are infauna, living within or partially within benthic substrate. Infauna often ingest, burrow in, and construct tube dwellings from sediment. They are the primary food source of many larger, bottom-feeding organisms, including some commercially important fish and crustaceans (Virnstein 1977, Arntz 1978). The majority of marine benthos undergo a meroplanktonic stage prior to settlement and metamorphosis to the juvenile and adult forms. Since STD results in near defaunation of large areas (Brinkhurst *et al.* 1987, Ellis *et al.* 1995a), the main mode of benthic recolonization during and after STD is through settlement of meroplankton from distant sources (Santos and Simon 1980a, Levin 1984, Taylor 1986).

While informative studies have been conducted on benthic recolonization during and after STD (Kathman *et al.* 1984, Ellis and Hoover 1990a, 1990b, Ellis *et al.* 1995a), these studies did not include experimental comparison of colonization in natural marine sediment and mine tailings. Without this comparison, it is difficult to attribute differences between benthic communities in tailings and in undisturbed, natural sediment to the presence of tailings. Characteristics of a benthic community during and after STD may reflect sediment characteristics, altered ecological succession attributable to the physical disturbance of STD, or natural variability (Zajac and Whitlatch 1982a, 1982b, Hughes 1984).

Experimental comparisons of different sediment types are often conducted in the laboratory using single species. While laboratory experiments are less expensive and time consuming, *in situ* experiments can be more informative. The advantage of an *in situ* experiment is added realism through incorporation of natural species assemblages, processes, and variability, as long as there is sufficient replication to produce statistically powerful results.

Study objective

The objective of this study was to determine if the presence of a tailings substrate would influence recolonization of benthos after STD. Macrofauna were selected as a component of the marine ecosystem that may be as likely as any other component to be sensitive to the presence of tailings substrate. An *in situ*, experimental approach was

used to isolate the influence of settled tailings on recolonization and to test a method that allows prediction of an aspect of benthic recovery prior to permitting an STD operation.

Materials and Methods

Study site

A study site was selected in Auke Bay, near Juneau, Alaska (134° 39.87'W, 58° 21.33'N, Figures 1 and 2). Auke Bay has been the subject of extensive physical and biological oceanographic research (Ziemann and Fulton-Bennett 1990). The climate of Auke Bay is temperate and maritime (Coyle and Shirley 1990). Numerous streams drain into Auke Bay, contributing to temperature and salinity stratification during the non-winter months. Bottom topography is irregular and the depth is approximately 40 m over most of the bay. The mean tidal range is approximately 4 m. Auke Bay is in the midst of several potential STD sites (Coldwell and Gensler 1993).

The study site in Auke Bay was selected because it was accessible, soft-bottomed, low in contaminants relative to industrialized areas and other sites in Auke Bay (Karinen 1985, Short *et al.* 1989), and nearly flat at 21 m, a depth that allowed work to be conducted using scuba. The site was approximately 3 km from a marina, the nearest potential source of substantial concentrations of contaminants. A mooring buoy was put in place at the site.

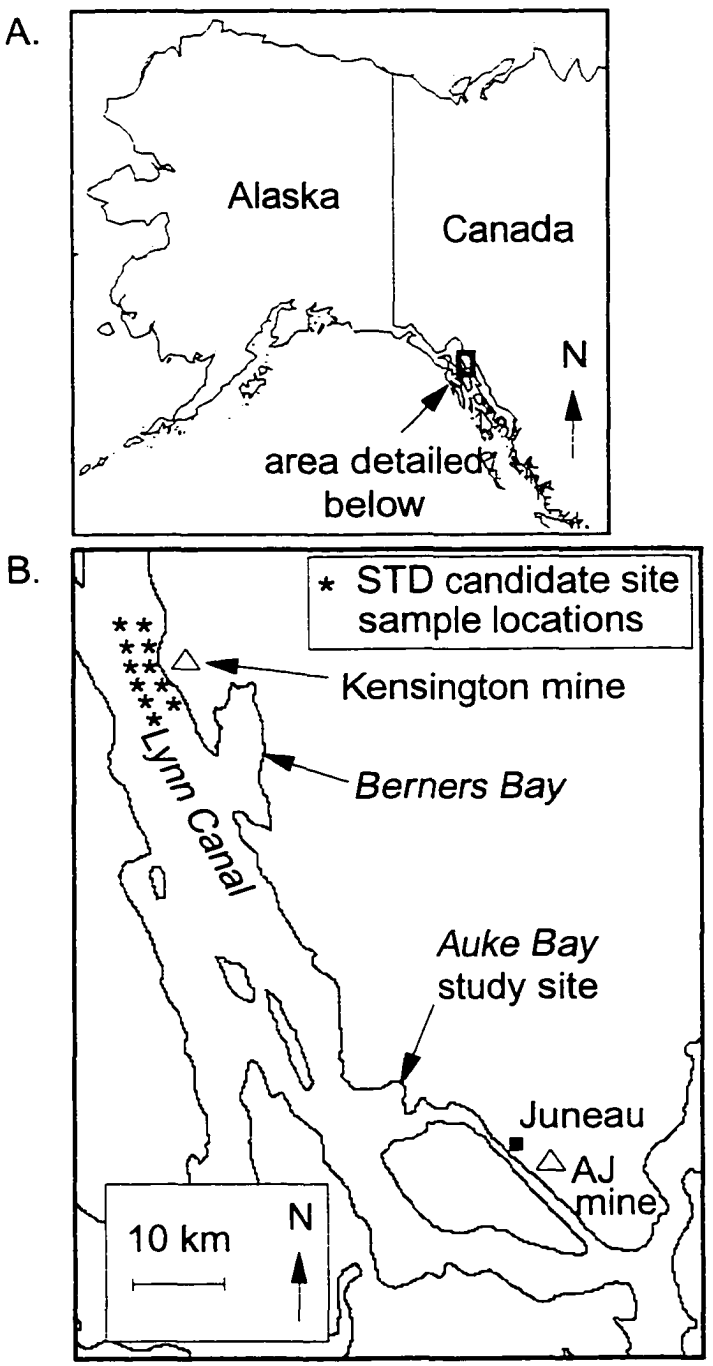

Figure 1. Locations of Auke Bay (site of colonization experiment), the Kensington Mine, STD candidate site samples, and the AJ Mine, another STD candidate. STD candidate site sample locations are approximate. Refer to Appendix 1 for exact locations. The average depths of the 6 sample locations nearest the center of Lynn Canal and the 5 sample locations nearest the east shore were 306 and 187 m, respectively.

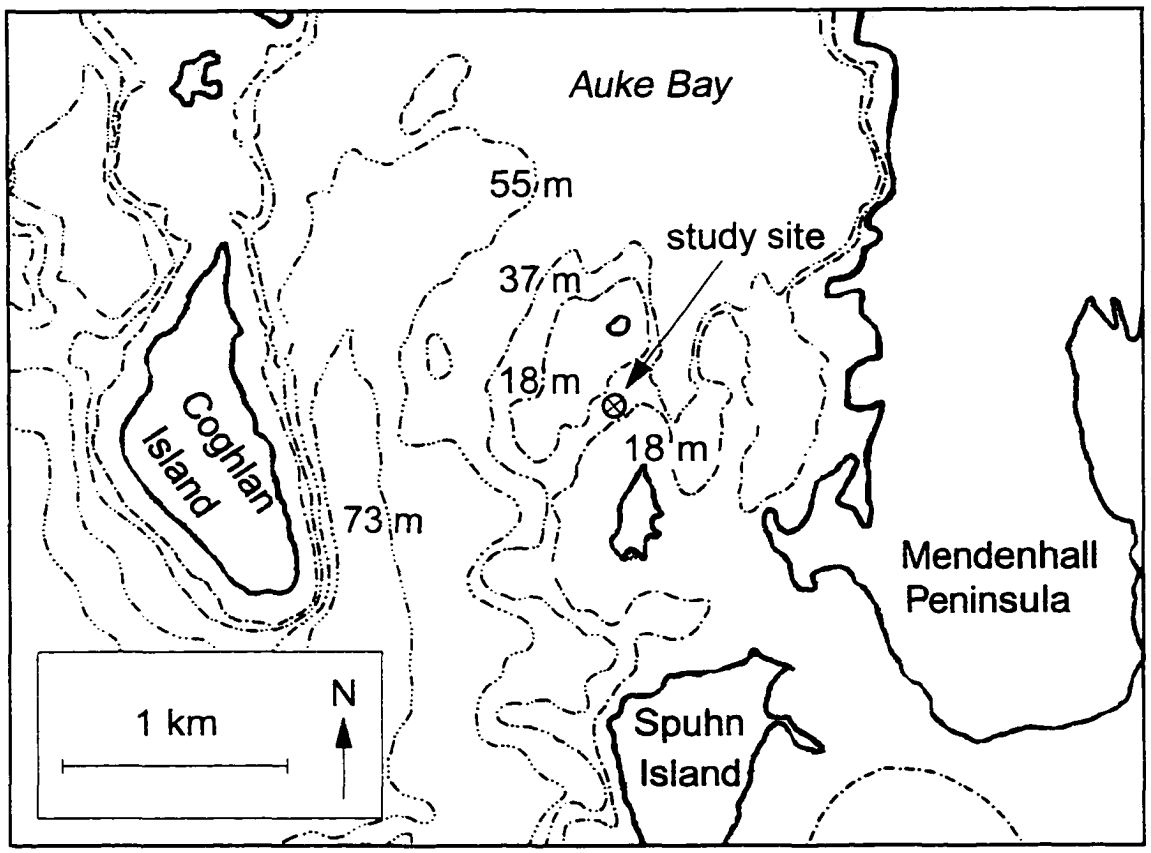

Figure 2. Depth contours of Auke Bay in the vicinity of the site of the colonization experiment. The islands to the north and south of the study site were Battleship Island and Suedla Island, respectively. Reference sediment was collected immediately northeast of the study site.

Experimental sediments

Approximately 1500 l of bottom sediment were collected during August 27-28, 1994 with a dredge near the study site for use as reference sediment (Figure 2). The sediment was processed to remove resident organisms, increase homogeneity, and approximate the grain size of the tailings. Processing consisted of freezing the sediment for 1 wk at -20°C, allowing it to become anoxic for 1 wk at room temperature, suspending it in freshwater, sieving it through 1 mm mesh to remove large particles, suspending it in sand-filtered seawater, and allowing it to settle for 1 d. The seawater was decanted and the sediment was mixed, placed in colonization trays, and refrigerated at 4°C for 6 wk. The trays of reference sediment were frozen at -20°C for 1 d prior to placing them *in situ* at the study site.

Tailings were produced by N.A. Degerstrom, Inc., Spokane, Washington, USA, from a representative sample of ore from the proposed Kensington gold mine, located 72 km north of Juneau, Alaska (Figure 1). The Kensington mine has been previously identified as a candidate for STD (Coldwell and Gensler 1993). Potassium amyl xanthate and methyl isobutyl carbinol were used as reagents in a pilot scale froth flotation process. The tailings used in this study were from the froth flotation process and did not include tailings from mineral concentrate processing. Tailings from froth flotation are of greater volume and lower metal content than tailings that result from processing mineral concentrate for gold. Mineral concentrate is often transported for processing elsewhere.

Tailings were shipped in a plastic lined drum and received at the Juneau Center, School of Fisheries and Ocean Sciences, University of Alaska Fairbanks on October 27, 1994. The drum of tailings was stored outside for 3 wk and then stored at room temperature for 6 d. During the outside storage period, the average daily temperature was 1.1°C, ranging from -4.4 to 5.6°C (National Weather Service, Juneau Forecast Office). After this storage period, 2 parts tailings were suspended in 5 parts sand-filtered seawater, by volume, to mimic dilution and particle separation during STD. Water and remaining suspended tailings were decanted after 20 min and the settled tailings were placed in colonization trays. The trays of tailings were frozen at -20°C for 1 d prior to placing them *in situ* at the study site.

The loss in weight of dry sediment (dried to constant weight at 60°C) after combustion at 475°C for 2 h was used as an estimate of organic content (Buchanan 1984). Grain size distribution was determined by weighing the sediment retained on a graded series of sieves (Buchanan 1984) followed by analysis of the settling velocity of the particles passing through a 75 μm sieve (American Society for Testing and Materials 1990).

Porosity was determined by suspending reference sediment or tailings in seawater to obtain approximately equal, constant, settled volumes in 100 ml graduated cylinders. Wet weight was determined after decanting overlying seawater. The sediment was repeatedly suspended in distilled water, allowed to settle, and decanted until the salinity of the decant water was < 5 ppt. The sediment was then

dried at 60°C to constant weight and percent porosity (P) was estimated by the equation:

$$P = \frac{W - D}{100(1.025V)}$$

where W = wet sediment weight, D = dried sediment weight, 1.025 = density of seawater (g/ml), and V = the settled volume of saturated sediment (adapted from Twenhofel and Tyler 1941).

Experimental design

Polyethylene colonization trays (8 cm deep, 15 cm diameter) were filled to 7 cm depth with reference sediment or tailings and placed within larger trays (10 cm deep, 28 cm diameter) (Figure 3). The space in the larger tray, surrounding the inner tray of reference sediment or tailings, was filled to 7 cm depth with reference sediment (439 cm^2 outside of inner tray). Lids were placed on the inner and outer trays prior to freezing and underwater placement at the study site.

The reference sediment in the outer portion of reference trays was intended to be compared to the reference sediment in the outer portion of tailings trays in an attempt to detect avoidance behavior. The hypothesis was that if some species avoided the tailings, higher densities of those species may occur in the reference sediment surrounding tailings trays. The outer trays also served to increase isolation of the inner tray sediment from the ambient sediment, and

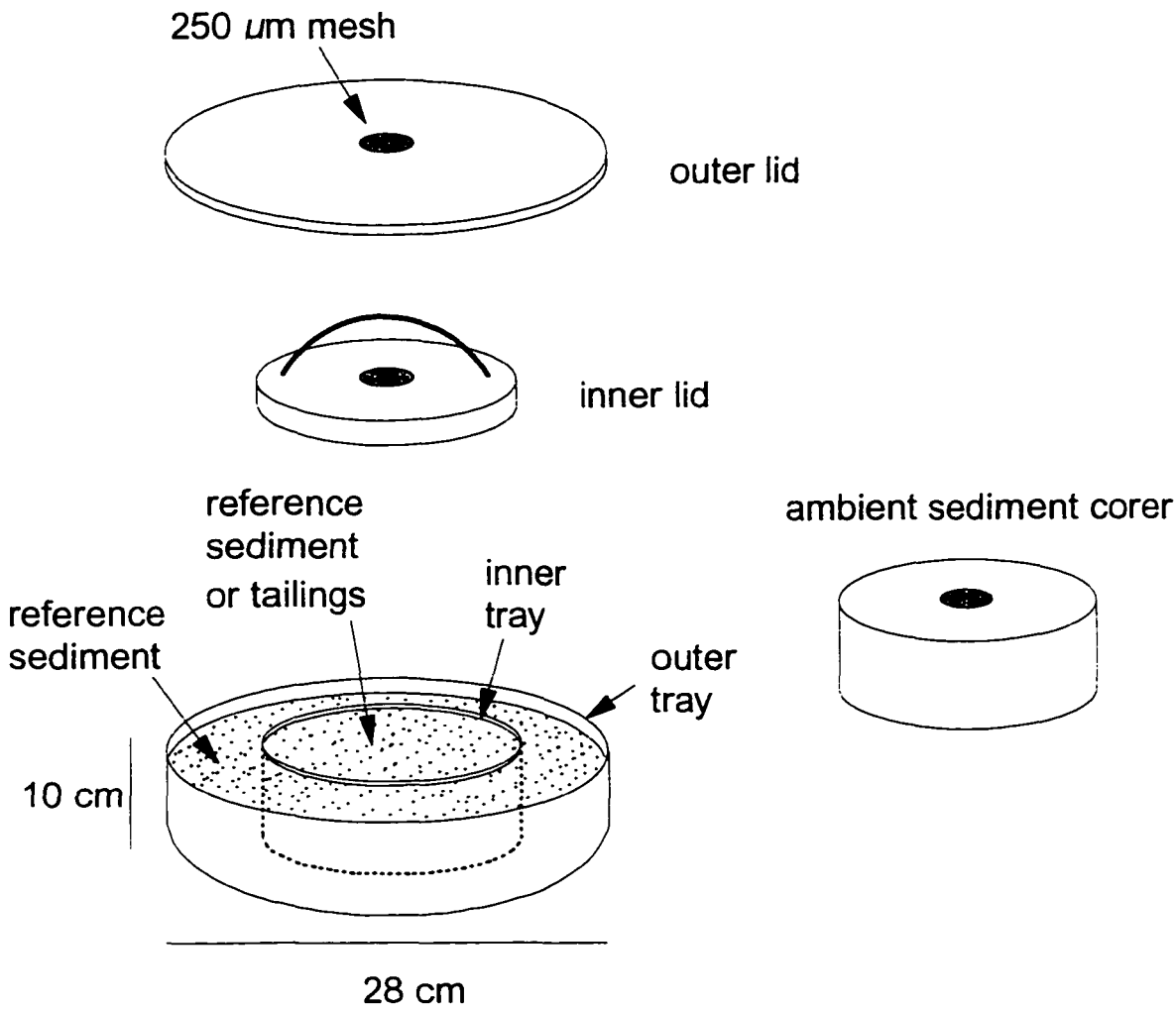

Figure 3. Trays, lids, and corer used for the colonization experiment. The corer was made from an inner tray. The trays and corer were made of polyethylene. The purpose of the mesh on the lids and corer was to allow water displacement during sampling.

possibly to reduce flow anomalies over the inner tray. The worth of the outer trays in detecting avoidance behavior could only be assessed if abundant taxa that were present in inner reference sediment trays were significantly less abundant in tailings. Due to a lack of differences between reference sediment and tailings assemblages, the outer tray sediment was not processed.

Sediment traps were placed *in situ* to determine the depth of sediment that accumulated on the reference sediment and tailings during the experiment. Sediment traps consisted of colonization trays filled with concrete, rather than sediment, to within 3 cm of the tray top. This design was used to minimize hydrodynamic bias that may have existed between the colonization trays and conventional sediment traps.

The trays of frozen reference sediment or tailings and the sediment traps were winched to the sea floor at the study site. Forty-eight trays of tailings and 48 trays of reference sediment were placed at 21 m depth around the circumference of a 30 m diameter circle using scuba (Figure 4). Starting from an initial random point on the circle, the trays were systematically arranged in alternating pairs of each sediment type, forming two concentric circles. At the same time, 2 sediment traps were placed in pairs at a random location within each quarter section of the circle, bringing the total to 104 trays. The trays were spaced an average of 1.8 m apart from tray center to tray center. Trays were not pressed into the ambient sediment since the objective was to isolate the tray sediment from the

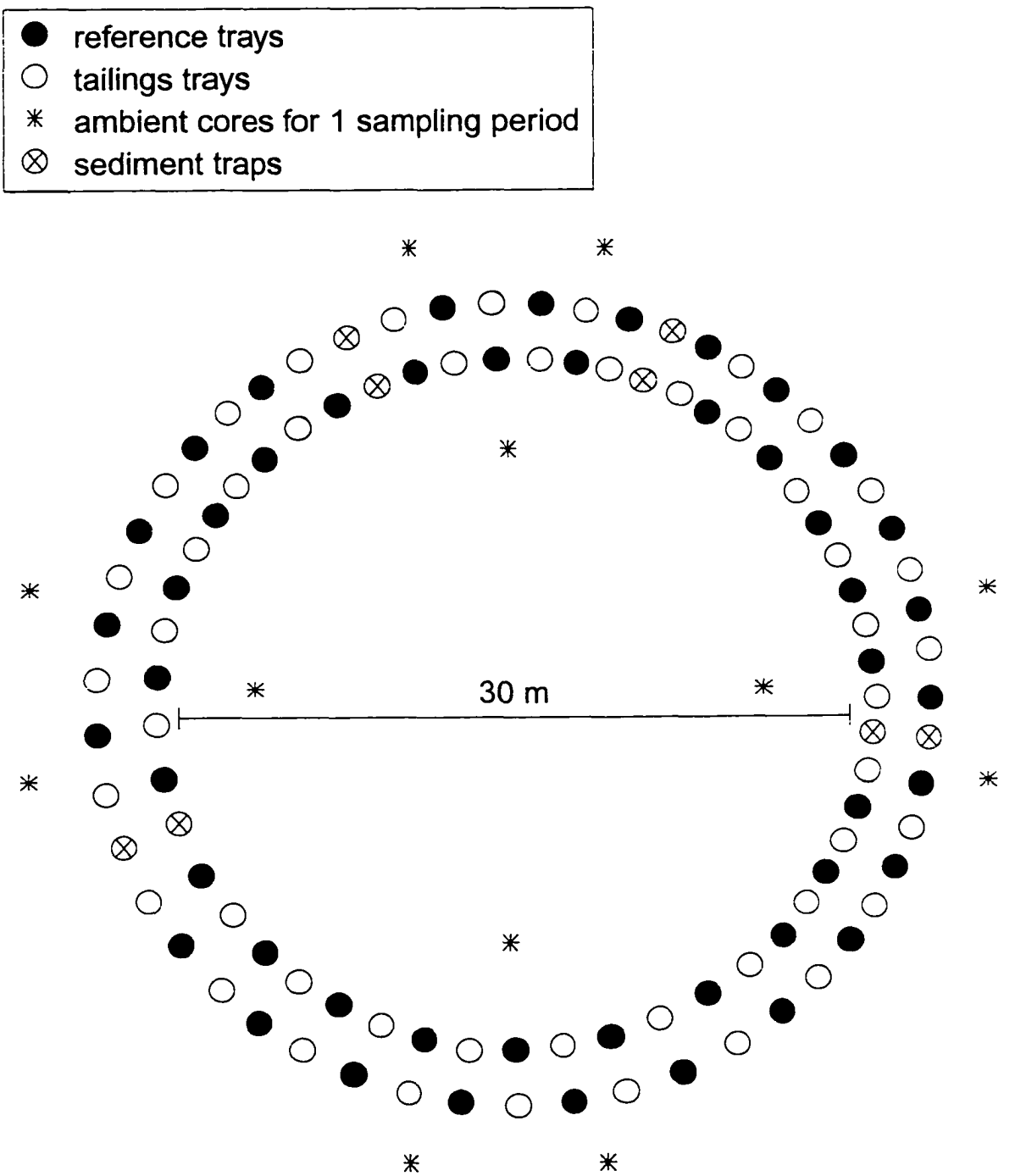

Figure 4. Top view of the arrangement of trays and ambient core locations for the colonization experiment. The trays are not to scale.

ambient macrofauna. The lids were removed from all trays on December 11, 1994.

Trays were retrieved during three periods: sampling period 1 (September 10-22, 1995); 2 (May 11-17, 1996); 3 (September 29-October 6, 1996), approximately 9, 17, and 22 months, respectively, after the lids were removed. Sampling dates were selected to be shortly prior to or shortly after the spring and summer periods of high meroplankton abundance in Auke Bay (Coyle and Paul 1990). The intent was for the spring samples (sampling period 2) to reflect macrofauna that were able to survive over winter in the trays, whereas the fall samples (sampling periods 1 and 3) were assumed to be comprised of proportionally more recently settled macrofauna (Bonsdorff and Österman 1985).

At least 12 each of reference sediment trays, tailings trays, and ambient sediment cores, and 2 sediment traps were collected during each sampling period. Immediately prior to retrieval, a lid was placed on the inner and outer tray. Both lids had a 2 cm hole for water displacement that was covered with 250 μm mesh (Figure 3). The inner lid was fitted with a handle so the lid could be carefully placed on the tray using one hand, minimizing disruption of the surficial sediment. A 3 cm wide aluminum band was attached to the lids of the inner trays. This band slid down the inner wall of the inner tray, preventing mixing of sediment between the inner and outer trays during retrieval. The larger lid was then placed on the outer tray and the tray was carried to the surface.

The objective in sampling the ambient sediment was to determine if the ambient assemblage converged over time with the tray assemblages, indicating attainment in the trays of the ambient successional stage. To maximize comparability between inner trays and ambient core samples, unused inner trays were used for coring (Figure 3). The corers, fitted with 250 μm mesh over a 2 cm hole in the bottom for water displacement, were gently placed, bottom up, on the sediment, pressed down, and pounded with a hammer until they were flush with the sediment surface. The surrounding sediment was then excavated and the cores and corers were sealed in plastic bags and transported to the surface.

Sample processing

Trays and cores were held at room temperature for 2 to 6 h to encourage a relaxed posture of organisms upon preservation. Samples were then sieved initially through 250 μm mesh, and fixed and stained in 10% buffered formalin with rose bengal. The outer and inner trays were processed separately. Each fixed sample was resieved through 500 μm mesh and preserved in 70% ethanol. Organisms retained on 500 μm mesh were sorted. The sediment on the sediment traps was allowed to resettle in the laboratory. The depth of sediment on each sediment trap was then measured at several regularly spaced points on each tray.

For the tailings trays from sampling period 2, a layer of sediment that had accumulated on the surface of the tailings was

processed separately from the underlying tailings. This was intended to allow estimation of the portion of macrofauna in direct contact with the tailings, as opposed to macrofauna that were restricted to the flocculent, surface layer of settled, natural sediment. Separation was achieved by gently rinsing the flocculent layer off of the tailings after tray retrieval until it was visibly apparent that the remaining sediment was comprised almost entirely of tailings. The tailings had settled firmly and did not rinse away easily. Since the reference sediment did not settle firmly, this technique would not have been effective for the reference sediment and was not performed. Except when stated otherwise, data from the flocculent layer and the underlying tailings were combined for data presentation and statistical analyses.

All macrofauna retained on a 500 μm sieve were sorted under a dissecting microscope at 64x and counted. Each individual was identified to species when practical using the keys of Fauchald (1977), Kozloff (1987), and the Santa Barbara Museum of Natural History (1996). A reference collection was maintained. A photographic album was also prepared along with a description of key features used to distinguish taxa. Three technicians participated in sorting of macrofauna by taxon from the samples. Identifications for all samples were confirmed by a single technician. Identifications of abundant polychaete species were confirmed by L. Harris, Curator of Polychaetes at the Los Angeles County Museum, Los Angeles, California, USA. Identifications of abundant bivalves were confirmed by T. Rice,

Curator of the Sea and Shore Museum, Port Gamble, Washington, USA.

Biomass was determined for the combined individuals of each taxon from each sample. Macrofauna were placed on pre-weighed foil, dried to constant weight at 60°C, combusted at 550°C for 5 h and re-weighed. The loss in weight after combustion was used as an estimate of ash-free dry weight biomass (Crisp 1971). Ten samples of each sediment type were processed for each sampling period, for a total of 90 samples.

Sampling of macrofauna at a candidate STD site

Benthic grab samples were collected from an area of Lynn Canal, adjacent to the Kensington mine (Figure 1). The purpose was to compare the taxa at a location that could conceivably receive the tailings used in this study to the taxa in the Auke Bay samples. Grab samples were collected because the depths at this candidate STD site exceeded scuba limitations.

On August 21, 1996, 0.05 m^2 samples were collected using a van Veen clam-shell type sampler at equally spaced intervals along two 7.7 km long transects running parallel to shore (Figure 1, Appendix 1). Five samples were collected from transect 1, nearest shore, at an average depth of 187 m (range = 23 m) and six samples were collected from transect 2, near the center of Lynn Canal, at an average depth of 306 m (range = 8 m). Sample size was uneven due to loss of a sample. Transect 1 was at the base of a steep slope that continued up to the shoreline on the east side of Lynn Canal. Transect 2 was at the

deepest portion of that section of Lynn Canal. Four cores were collected from the surface of each grab sample to a depth of 7 cm and combined. The combined cores equalled the depth, volume, and surface area of the inner colonization trays and ambient sediment cores. The samples were processed in the same manner as the Auke Bay samples except biomass was not determined since many of the specimens from the Lynn Canal samples were retained for a reference collection.

Data analysis

Assemblage attributes among sediment types for each sampling period were analyzed using single classification, model I ANOVA. When differences were significant ($p \leq 0.05$), Tukey's tests were conducted for the three pairwise comparisons. Assemblage attributes that were analyzed in this manner included total abundance, total biomass, number of taxa, average size of an individual (biomass/abundance), average size of individuals of the 4 most abundant taxa, abundance by feeding type, and abundance by sediment association type. Abundance and biomass data were transformed to $(x + 0.5)^{.5}$ and $\log_{10}(x + 1)$, respectively, prior to analysis to increase normality, homoscedasticity, and additivity (Zar 1996). Back transformed means and 95% confidence intervals were reported. These same statistical methods were used to analyze differences in sediment organic content and porosity. Organic content and porosity were transformed to the arcsin of the square root to better meet the assumptions of ANOVA.

Correspondence analysis was used to graphically display the

relationship among the three sediment types for each sampling period using raw abundance in a taxa by sample matrix. The distance between points, each point representing a sample, reflected the overall similarity in the taxonomic composition, weighted by abundance (Greenacre and Underhill 1982, Greenacre and Vrba 1984). As such, large differences in abundance of a few numerically dominant taxa, or numerous differences in the presence of rare taxa, could each have contributed to discriminating between samples. Sample identification was used only to identify the sediment type of the plotted points. Otherwise, no distinction of sediment type was made in the analyses. After the correspondence analysis for each sampling period, 95% confidence regions were generated around the plotted points for each sediment type. All statistical analyses were performed using SYSTAT 7.0 for Windows (SPSS Inc. 1997).

For classification of taxa into functional groups, published literature and taxonomic keys were searched for general ecological descriptions of each taxon. Feeding types included subsurface deposit, surface deposit, suspension, carnivore, or combinations of these feeding types for taxa that switch feeding strategies. Herbivores and scavengers were grouped as *other*, due to low abundance. Sediment association types included burrowers, tube builders, and surface dwellers. When species information was not found, generalizations pertaining to genus or family were used, provided the description pertained to soft-bottom dwelling members of the taxon. Taxa that were described as burrowing in addition to displaying

surface association or errant behavior were classified as burrowers. Taxa for which no functional information was found were grouped as *unclassified*.

The relationships between statistical power and minimum detectable difference and between power and sample size were based on the formula:

$$\delta = \sqrt{\frac{2ks^2\Phi^2}{n}}$$

where δ is the minimum detectable difference, k is the number of groups (3), s^2 is the mean square error using raw data, n is the sample size (10), and Φ is a quantity incorporating k, n, and the probabilities of committing a Type I and Type II error (Zar 1996). The quantity δ was divided by the raw reference mean and multiplied by 100 to obtain the minimum detectable difference as a percent of the reference mean.

Taxa richness was compared using rarefaction methodology. The expected number of taxa (E) in a random sample of n individuals was calculated and plotted as taxa accrual curves using the formula:

$$E(n) = \sum_{i=1}^{k} \left[1 - \left(1 - \frac{t_i}{\sum_{i=1}^{k} t_i}\right)^n\right]$$

where k is the number of taxa in a sample and t_i is the number of individuals of taxon i (Smith and Grassle 1977).

Results

Sediment analysis

The organic content of tailings was significantly lower ($p \leq 0.05$) than that in ambient sediment and reference sediment (Table 1). The porosity of tailings was significantly lower than that of reference sediment. Porosity of ambient sediment was not determined since it was assumed to be affected by the disruption of the sampling process. A notable difference between reference sediment and tailings that was not quantified was compaction. Using a finger, settled, saturated reference sediment was easily penetrated whereas settled, saturated, tailings were impenetrable.

Compared to tailings, reference sediment was comprised of proportionally more particles ≤ 40 μm (Figure 5). Ambient sediment had a coarser and a finer component than reference sediment and tailings. The coarser component of ambient sediment consisted of shell fragments and particles sometimes exceeding 5 cm in diameter. These coarse particles were removed from ambient sediment during the sieving process while preparing reference sediment. The finer component of ambient sediment was removed with the decant water during the homogenation and rinsing procedures while preparing reference sediment.

Table 1. Organic content and porosity of reference sediment, tailings, and ambient sediment from the colonization experiment. Reference sediment and tailings were analyzed prior to *in situ* placement. Ambient sediment data are for cores collected during sampling period 2. Values are means (standard error). Means that do not share a superscripted letter were significantly different (Tukey's, $p \leq 0.05$). Sample size = 3 for organic content and 4 for porosity.

	Sediment type		
Characteristic	Reference	Tailings	Ambient
Organic content (%)	2.06^{a} (0.15)	0.27^{b} (0.02)	1.52^{a} (0.06)
Porosity (%)	78^{a} (0.5)	51^{b} (3.3)	nd

nd = not determined

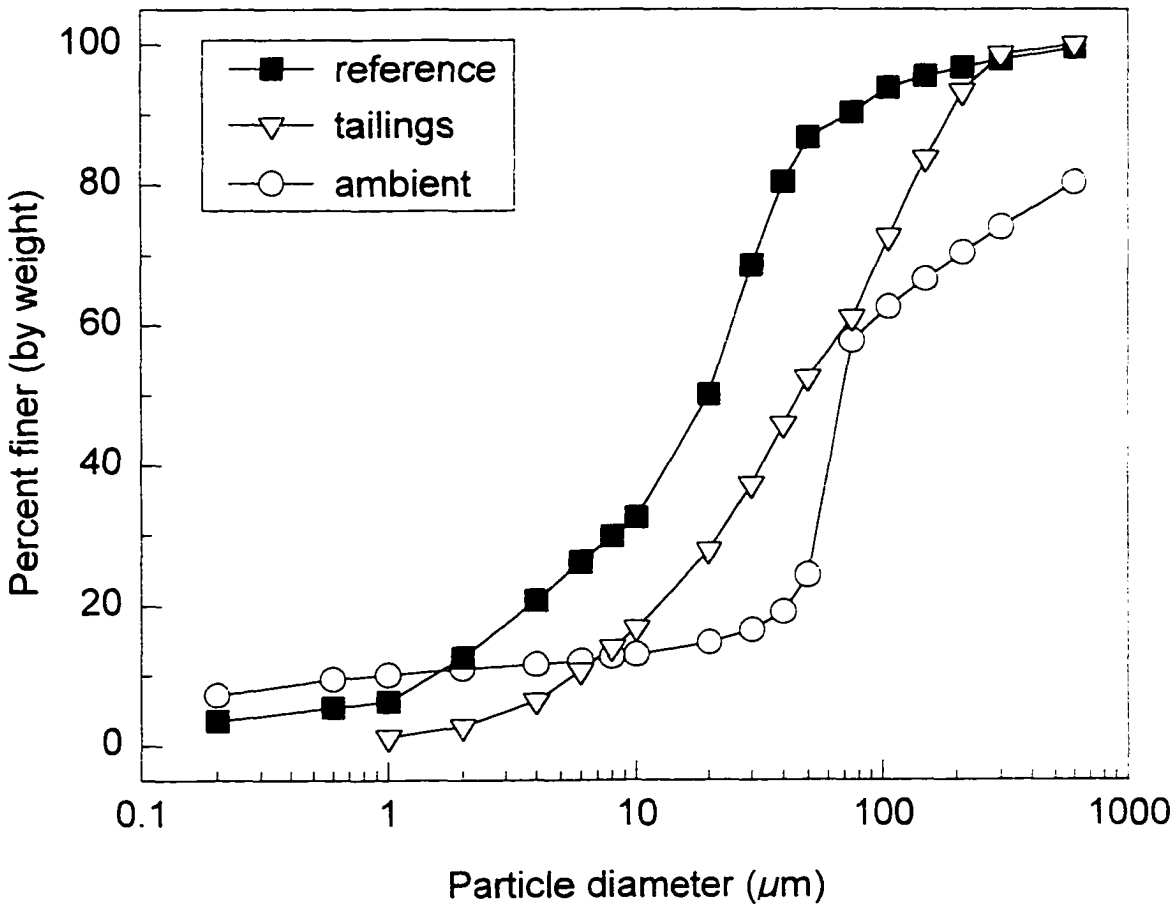

Figure 5. Grain size distributions of reference sediment and tailings prior to *in situ* placement, and ambient sediment from sampling period 1.

Whole assemblage attributes

All results are for the inner trays. The outer tray sediment was not processed. Total abundance was significantly lower in tailings than in reference sediment only for sampling period 2 (Figure 6A, Appendix 2). There were no significant differences between reference sediment and tailings in total biomass (Figure 6B). The relative differences between reference sediment and tailings were greater for total abundance than for total biomass for sampling periods 2 and 3 because the average size of an individual (biomass/abundance) in tailings was slightly larger than in reference sediment (Figure 6C). Differences between reference sediment and tailings in biomass/abundance and number of taxa were insignificant for each sampling period (Figures 6C and D).

Total abundance was significantly lower in ambient sediment than in tailings or reference sediment for sampling period 2 (Figure 6A). Total abundance in ambient sediment doubled from sampling period 1 to 3, compared to an increase in total biomass of 32% (Figures 6A and B). The average biomass per individual (biomass/abundance) in ambient sediment was smallest during sampling period 3 (Figure 6C). This explains why abundance increased more than biomass during the study. The number of taxa was significantly lower in ambient sediment than in reference sediment and tailings for all sampling periods (Figure 6D).

The minimum detectable difference as a percent of the reference mean, progressing from lowest to highest was generally: number of taxa; total abundance; total biomass; biomass/abundance (Figure 7).

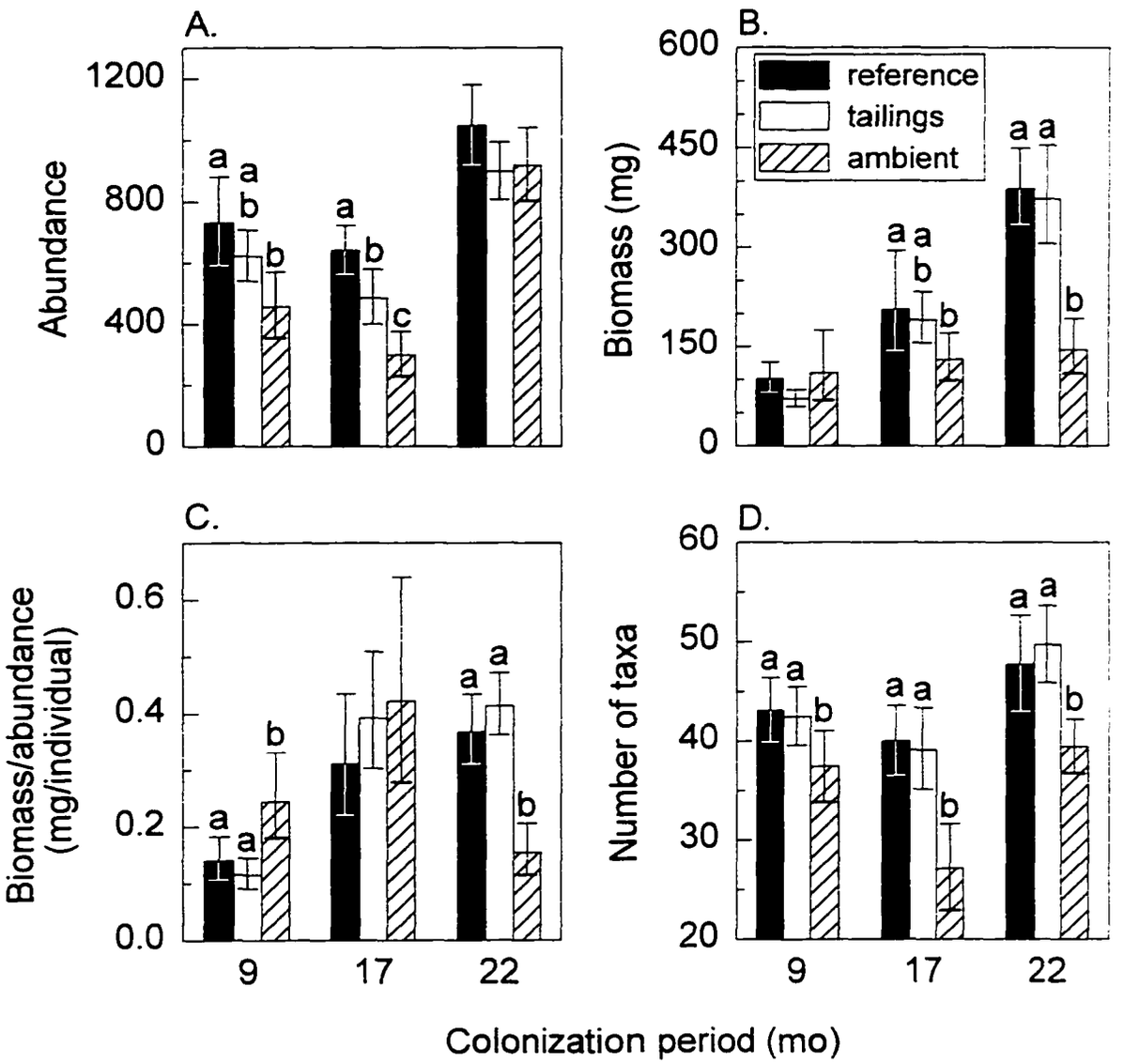

Figure 6. Whole assemblage attributes of macrofauna from the colonization experiment. The 9, 17, and 22 month colonization periods were sampling periods 1, 2, and 3, respectively. Biomass is based on ash-free dry weight. Bars represent means and 95% confidence intervals for entire samples (177 cm^2). Groups of bars for each attribute within a sampling period that have letters but do not share a letter were significantly different (Tukey's, $p \leq 0.05$).

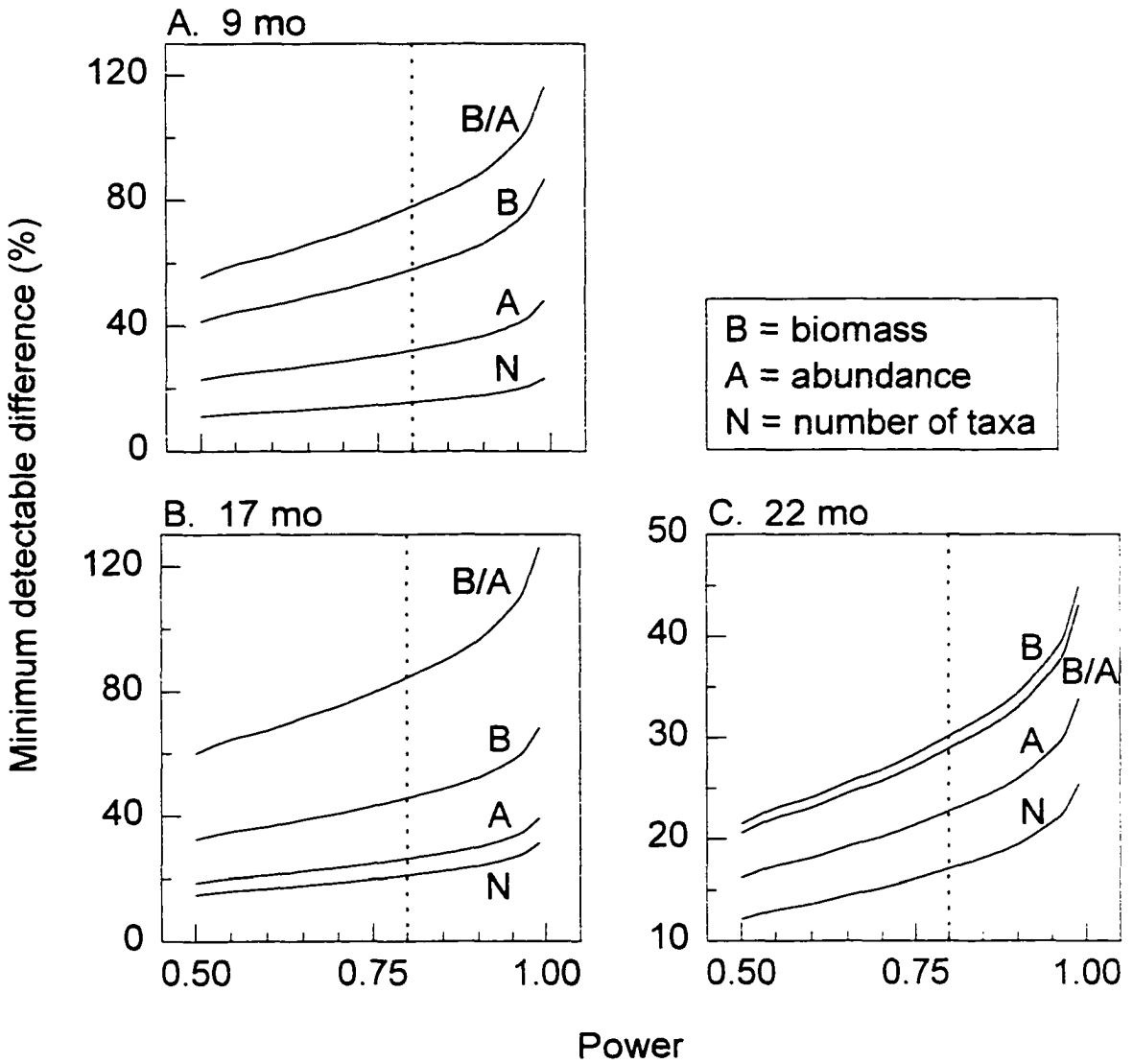

Figure 7. Minimum detectable difference of ANOVA as a percent of the reference mean for whole assemblage attributes of macrofauna from the colonization experiment (α = 0.05, n = 10). The 9, 17, and 22 month colonization periods were sampling periods 1, 2, and 3, respectively. Vertical dashed lines indicate statistical power (1-β) of 0.8.

The sample size of 10, chosen for this study, was sufficient to nearly maximize the ability to detect differences in number of taxa (Figure 8). Power for the other whole assemblage attributes would have been substantially increased with a larger sample size, with the exception of abundance for sampling period 3, which neared the asymptote at n = 10. Power was greatest for all whole assemblage attributes for sampling period 3. This was due, in part, to presenting the minimum detectable difference as a percent of the reference mean and the fact that reference sediment means were highest for sampling period 3 (Figure 6).

For any given number of individuals occurring in the three sediment types, taxa richness using rarefaction methodology was nearly equal or greater in tailings than in reference sediment, and lowest in ambient sediment (Figure 9). For each sediment type, taxa richness was lowest during sampling period 2.

The reference sediment and tailings assemblages for each sampling period were indistinguishable using correspondence analysis (Figure 10). The 95% confidence regions for reference sediment nearly contained the confidence regions for tailings for sampling periods 1 and 3. The tailings confidence region completely contained the reference sediment region for sampling period 2. The ambient sediment points were clearly separate from the reference sediment and tailings points for each sampling period. The percent of the variation explained by correspondence analysis ranged from 28 to 36 for dimension 1 (Figure 10). Dimension 2 explained 9% of the variation

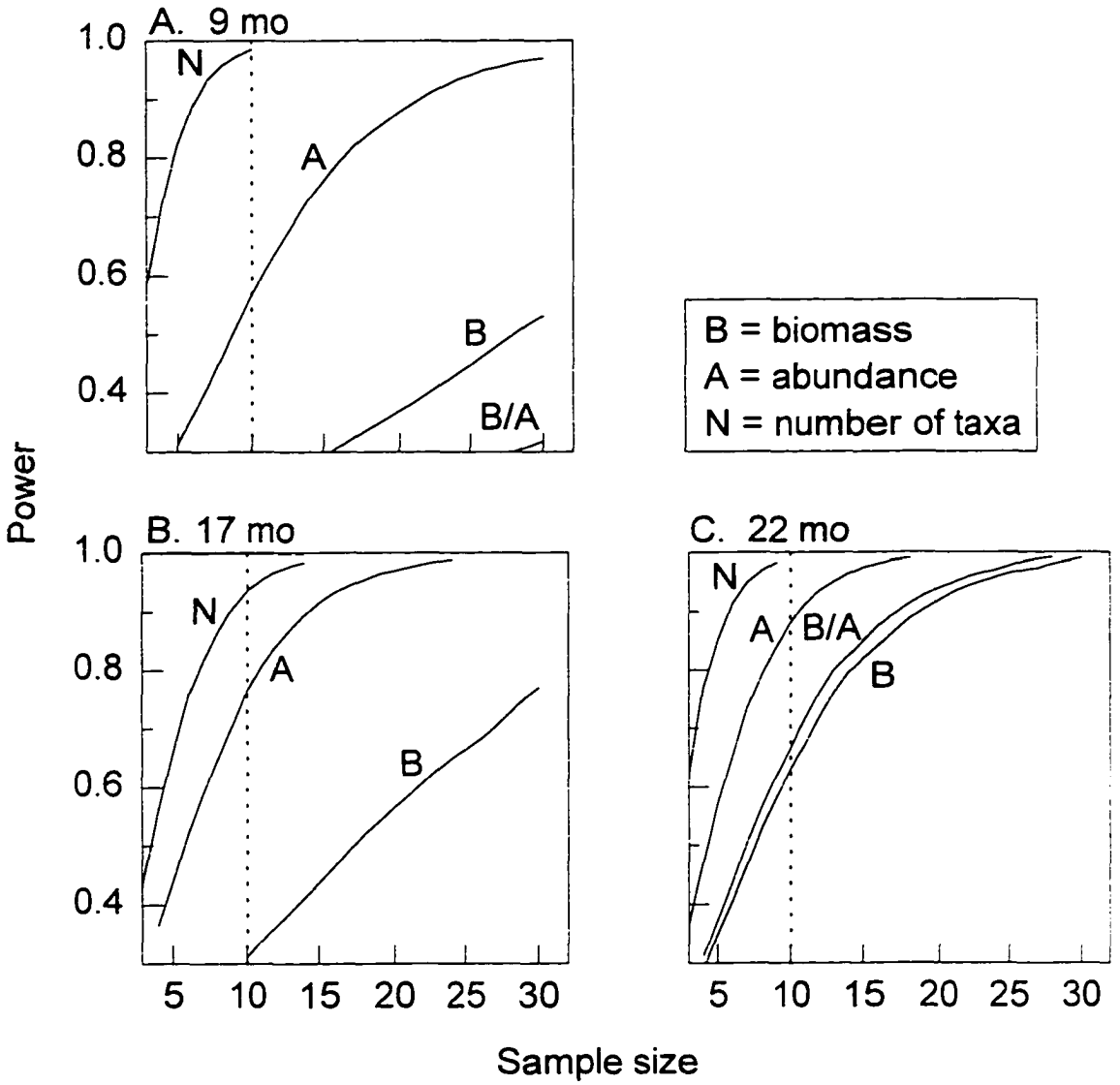

Figure 8. Relation of sample size and statistical power of ANOVA for whole assemblage attributes of macrofauna from the colonization experiment (α = 0.05, 1-β = 0.8, minimum detectable difference = 25% of reference mean). The 9, 17, and 22 month colonization periods were sampling periods 1, 2, and 3, respectively. B/A for 17 months was below the range of power that was included. Vertical dashed lines indicate the sample size used for this study (n = 10).

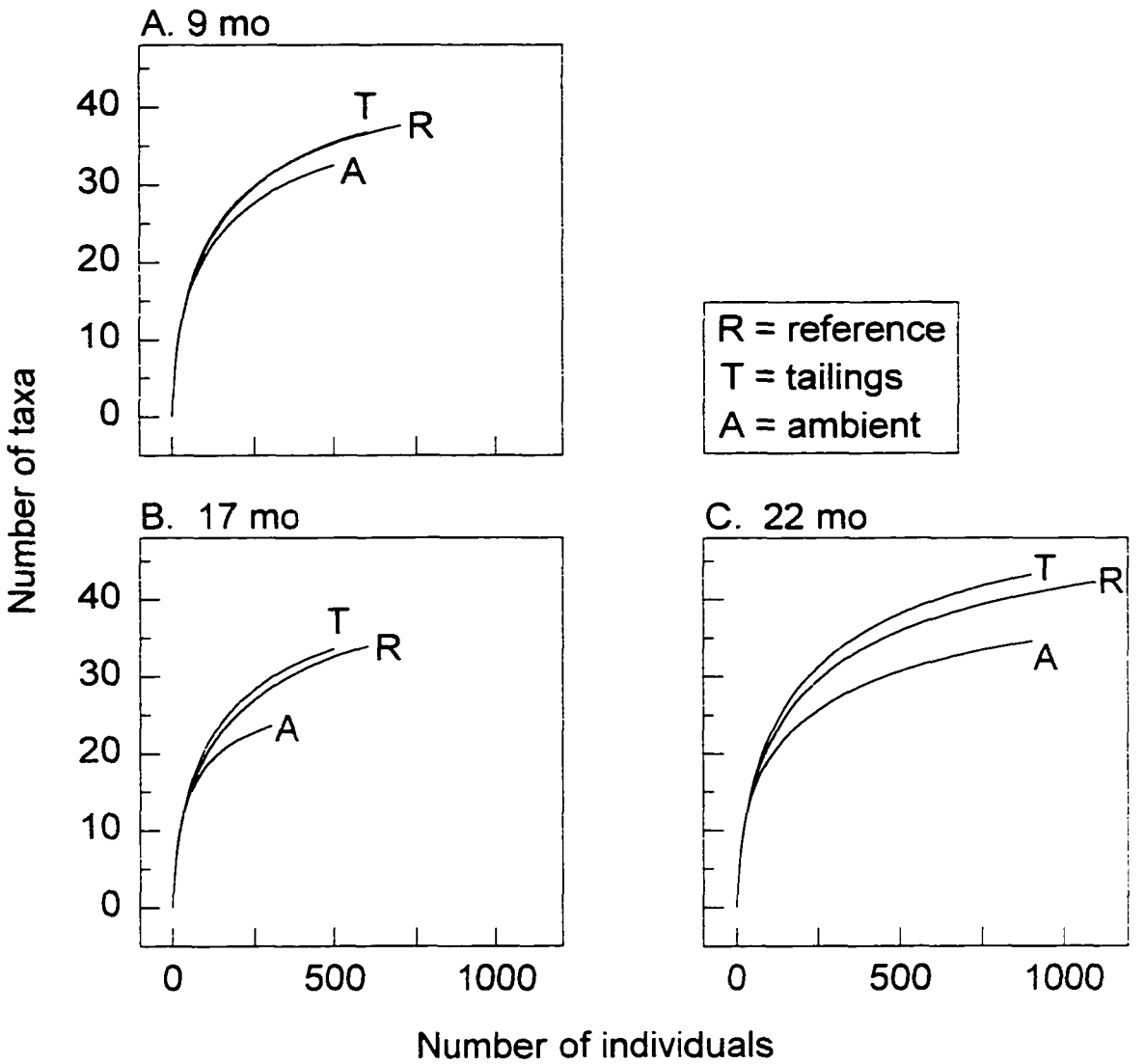

Figure 9. Taxa richness using rarefaction methodology of macrofauna from the colonization experiment. The 9, 17, and 22 month colonization periods were sampling periods 1, 2, and 3, respectively. Curves represent the expected number of taxa in a random sample of n individuals. The length of the curves was determined by the average total abundance (177 cm^2 samples) for each sediment type.

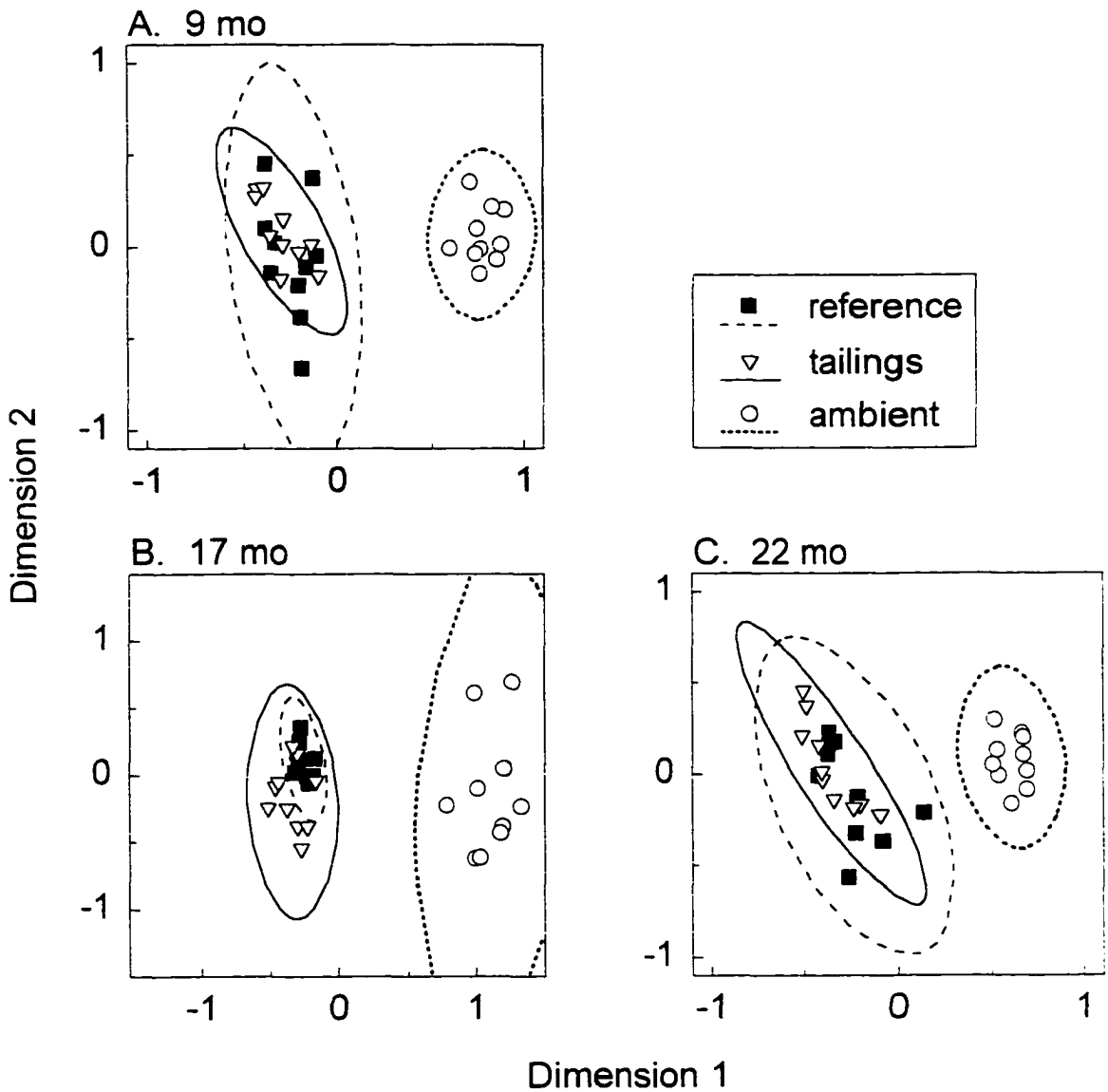

Figure 10. Relation of macrofaunal assemblages for each sediment type from the colonization experiment using correspondence analysis. The 9, 17, and 22 month colonization periods were sampling periods 1, 2, and 3, respectively. The distance between points indicates the overall similarity of taxonomic composition, weighted by abundance. Each point represents a replicate reference sediment tray, tailings tray, or ambient core. Replicates for each sediment type are surrounded by 95% confidence regions, generated after the analysis.

for each sampling period. The greatest contributions to both dimensions were differences in the abundance of abundant taxa, including *Mediomastus californiensis* and *Galowthowenia oculata*.

The majority of macrofauna from sampling period 2 were in direct contact with tailings, beneath the flocculent surface layer (Table 2). The abundance of macrofauna in the flocculent layer, as a percent of the total, was greater than biomass, reflecting the relatively small size of the surface fauna. Approximately 5, 6, and 11 mm of sediment had accumulated on the surface of the cement in the sediment traps for sampling periods 1, 2, and 3, respectively.

Species level comparisons

With the exception of unidentified nematodes, all of the numerically dominant taxa were polychaetes, each of a different family (Table 3). Other taxa that were commonly found in the trays and cores included amphipods, cumaceans, bivalves, and gastropods (Appendices 3 - 6). *M. californiensis* and *G. oculata* were the most abundant taxa in reference sediment and tailings, respectively, for each of the three sampling periods. Reference sediment and tailings shared the three most abundant taxa for each sampling period, although the rank order was different. These same three taxa ranged from the most abundant to the ninth most abundant in ambient sediment. In particular, *G. oculata was* less abundant in ambient sediment than in the trays for sampling periods 2 and 3.

When combined, the 9 taxa that comprised > 2% of the total

Table 2. Whole assemblage attributes of macrofauna from the colonization experiment in the flocculent surface layer and in the underlying tailings for sampling period 2 (SE, standard error, n = 10). Values in parentheses are percent of total. Percent of total was not calculated for number of taxa because the two sediment layers shared some taxa.

	Abundance		Ash-free dry weight biomass (mg)		Number of taxa		Biomass/abundance (mg/individual)	
Sediment layer	Mean	SE	Mean	SE	Mean	SE	Mean	SE
floc on tailings	75.5 (15.3)	13.4	5.3 (2.7)	1.72	17.7	1.21	0.051	0.020
tailings w/o floc	417.1 (84.7)	31.2	188.9 (97.3)	15.22	32.5	2.10	0.416	0.048

Table 3. Abundance (Ab.) and biomass (Bi.) of the most abundant taxa of macrofauna from the colonization experiment. The 9, 17, and 22 month colonization periods were sampling periods 1, 2, and 3, respectively. Values are percent of the total for each sediment type and sampling period. Taxa that comprised > 2% of the total abundance across sediment types and sampling periods were included. All taxa were polychaetes with the exception of Nematoda.

		Colonization period (mo)					
		9		17		22	
Taxon	Sediment type	Ab.	Bi.	Ab.	Bi.	Ab.	Bi.
Mediomastus californiensis	reference	25.2	1.9	30.2	2.4	22.6	2.2
	tailings	18.5	1.5	18.4	0.8	18.0	1.1
	ambient	14.6	1.3	17.9	0.9	10.2	0.8
Galathowenia oculata	reference	14.5	2.3	18.8	9.7	17.8	14.7
	tailings	20.5	3.3	26.6	10.8	22.0	15.7
	ambient	10.3	1.5	4.3	1.7	6.8	2.1
Prionospio steenstrupi	reference	7.8	4.4	4.1	0.8	18.3	2.3
	tailings	9.1	5.1	3.9	1.0	20.2	3.1
	ambient	13.2	1.7	12.4	1.6	24.3	4.5
Pholoe glabra	reference	8.6	0.7	8.5	0.5	4.9	0.8
	tailings	10.3	1.0	9.1	0.6	4.0	0.6
	ambient	9.3	0.7	3.9	0.5	7.3	1.2
Nephtys cornuta	reference	5.2	0.9	6.5	0.3	6.1	0.5
	tailings	3.7	1.6	8.2	0.5	3.5	0.3
	ambient	9.4	1.1	7.1	0.4	11.9	1.0
Lumbrineris luti	reference	1.4	13.6	2.0	7.0	3.3	8.4
	tailings	1.1	12.1	1.2	4.0	2.9	5.0
	ambient	10.0	31.2	16.2	36.1	7.6	33.1
unidentified Nematoda	reference	0.8	0.0	1.0	0.0	1.0	0.1
	tailings	0.8	0.0	0.6	0.0	0.8	0.0
	ambient	4.3	0.0	7.9	0.1	7.8	0.1
Glycinde polygnatha	reference	3.7	3.3	1.6	0.7	1.7	1.8
	tailings	4.9	2.4	1.8	1.0	1.8	1.3
	ambient	2.4	2.0	1.5	1.2	1.6	2.2
Clymenella torquata	reference	1.0	0.3	6.2	2.3	1.8	1.2
	tailings	1.5	0.2	4.2	0.5	2.3	0.7
	ambient	0.4	0.2	2.1	0.3	0.4	0.1

abundance across sediment types and sampling periods (Table 3) comprised 68 to 79% of the total abundance by sampling period and sediment type. These same taxa comprised 19 to 45% of the total biomass by sampling period and sediment type. The disparity between the percentage of total abundance and biomass indicated that individuals of the most abundant taxa were smaller than the average-sized individual. This finding clearly applied to *M. californiensis*, whose percentage of total abundance exceeded its percentage of total biomass by > 10x. In contrast, the contribution of *G. oculata* to total biomass approached that of total abundance by sampling period 3. Across sampling periods and sediment types, the taxa that had the greatest total biomass, in rank order were: *Laonome kroyeri; Lumbrineris luti; Ophelina acuminata; G. oculata*. These 4 taxa were polychaetes of greater than average size and abundance.

Individuals of 3 of the 4 most abundant taxa were significantly larger in ambient sediment than in the trays for sampling period 1 (Figure 11, Appendix 7). By sampling period 3, these same taxa were significantly smaller in ambient sediment than in the trays. The 4 most abundant taxa were generally of similar size in reference sediment and tailings with some exceptions. *M. californiensis* was significantly larger in reference sediment than in tailings for sampling periods 2 and 3, and *Prionospio steenstrupi* was significantly larger in tailings relative to reference sediment for sampling period 3. Minimum detectable differences for biomass/abundance of numerically dominant taxa as a percent of the reference mean ranged

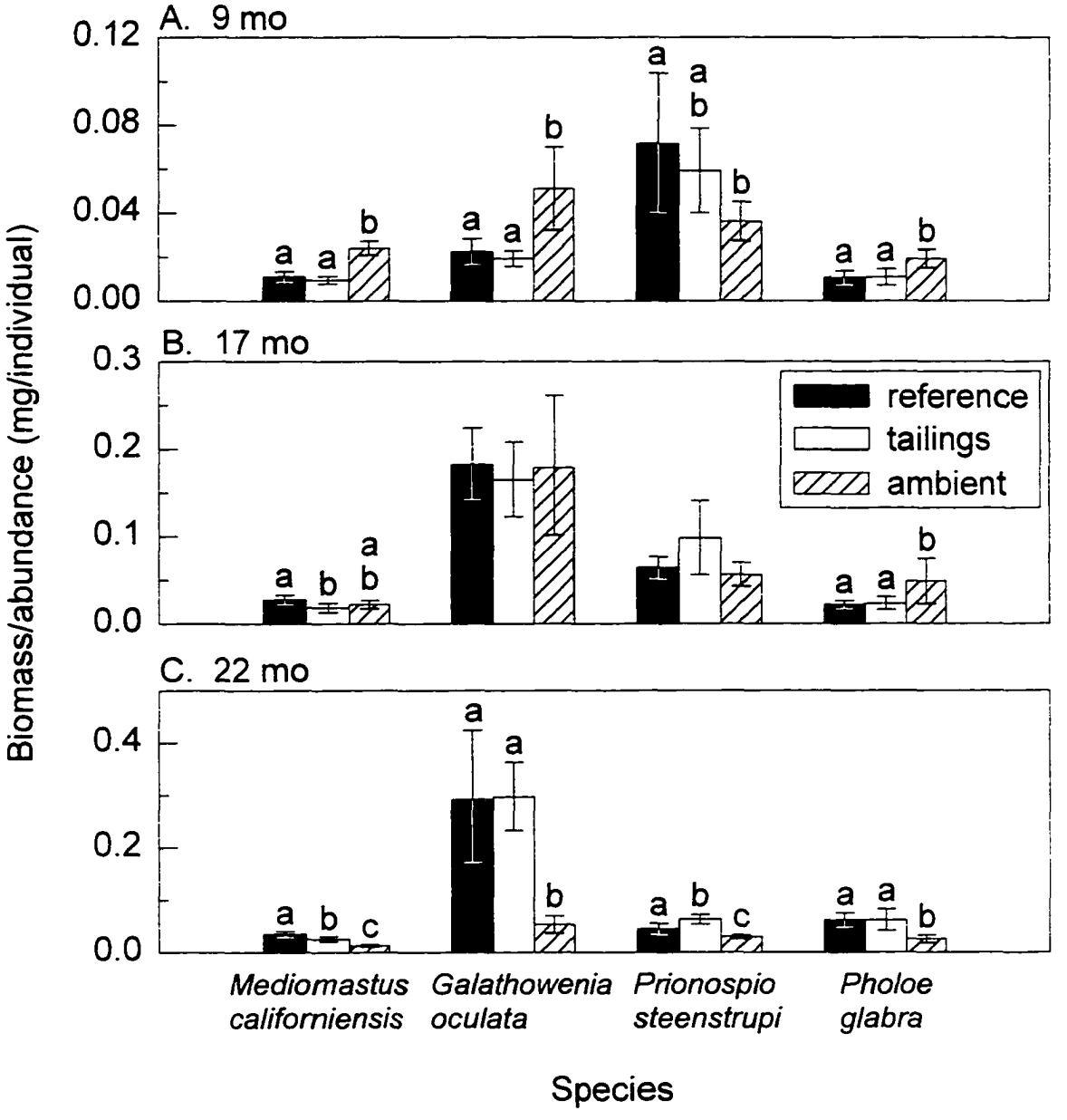

Figure 11. Average size (biomass/abundance) of numerically dominant taxa of macrofauna from the colonization experiment. The 9, 17, and 22 month colonization periods were sampling periods 1, 2, and 3, respectively. Biomass is based on ash-free dry weight. Bars represent means and 95% confidence intervals. Groups of bars for each species within a sampling period that have letters but do not share a letter were significantly different (Tukey's, $p \leq 0.05$).

from 26 to 108 (α = 0.05, 1-β = 0.8).

Functional groups

Among the most abundant taxa (Table 3), all of the feeding and sediment association types that were specified in Figures 12 and 13 were represented, but no combination of feeding type and sediment association type prevailed (Appendices 8 and 9). Burrowing taxa were most common, including *M. californiensis*, a subsurface deposit feeder (Barnes 1987, Kalke and Montagna 1991, Lastra *et al.* 1991). Total abundance of tube builders was comparable to burrowers, although only 3 of the abundant taxa were tube builders, compared to 5 abundant burrowing taxa. The only abundant surface dweller was *Pholoe glabra*, a carnivore (Fauchald and Jumars 1979). The most abundant taxon in tailings, *G. oculata*, is a tube building species that switches between surface deposit and suspension feeding (Fauchald and Jumars 1979, Barnes 1987).

The distribution of abundance by feeding type was similar for reference sediment and tailings compared to ambient sediment (Figure 12). Differences in abundance of subsurface deposit and surface deposit/suspension feeders were attributable mainly to *M. californiensis and G. oculata*. Differences in the abundance of carnivores reflected the abundance of *P. glabra* and *Nephtys cornuta*.

Burrowers were generally less abundant in tailings than in reference sediment (Figure 13), owing largely to lower abundance of *M. californiensis* in tailings. Differences in the abundance of surface

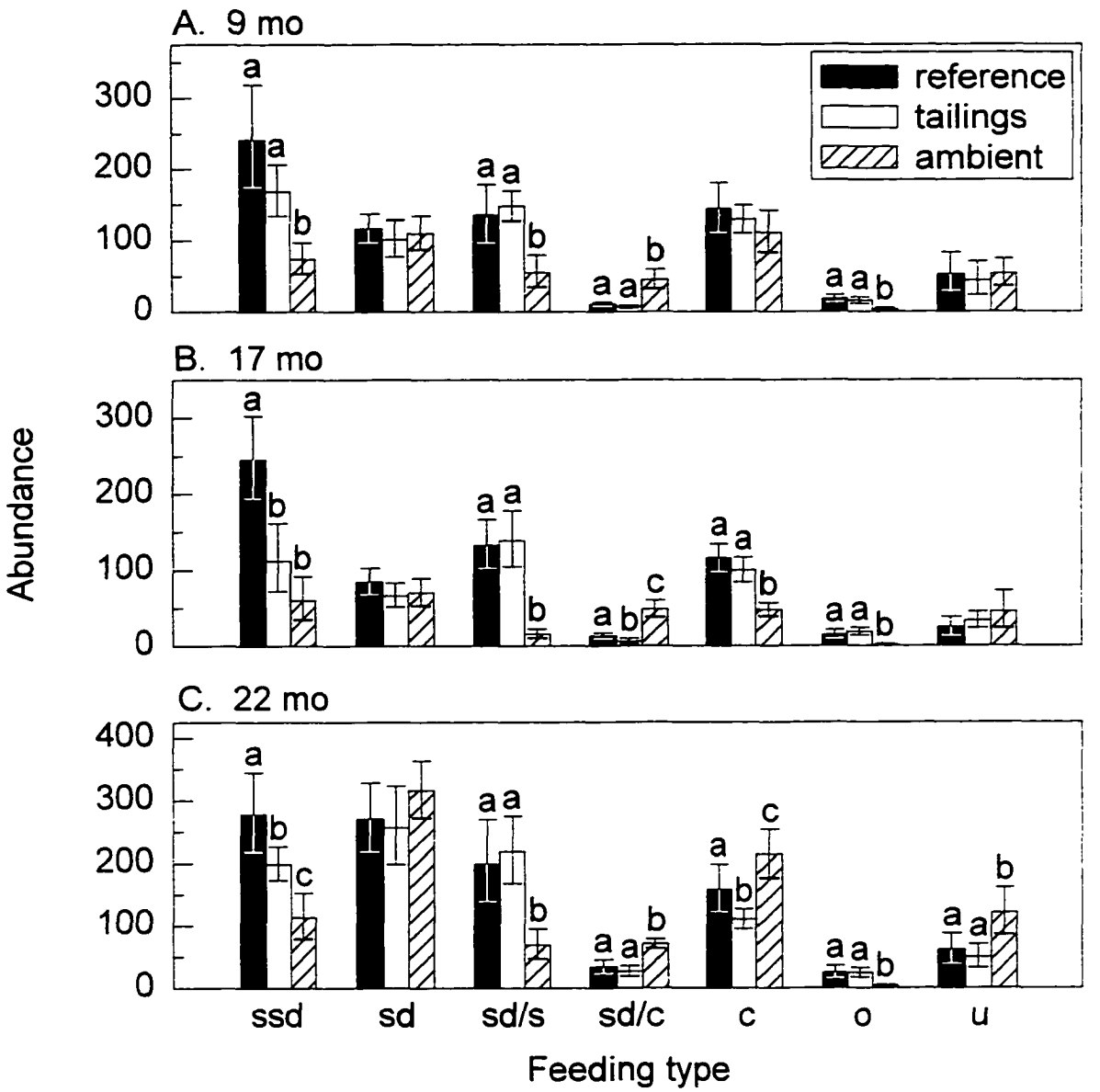

Figure 12. Abundance by feeding type of macrofauna from the colonization experiment. The 9, 17, and 22 month colonization periods were sampling periods 1, 2, and 3, respectively. Bars represent means and 95% confidence intervals for entire samples (177 cm^2). Feeding type: ssd, subsurface deposit; sd, surface deposit; sd/s, surface deposit/suspension; sd/c, surface deposit/carnivore; c, carnivore; o, other; u, unclassified. Groups of bars for each feeding type within a sampling period that have letters but do not share a letter were significantly different (Tukey's, $p \leq 0.05$).

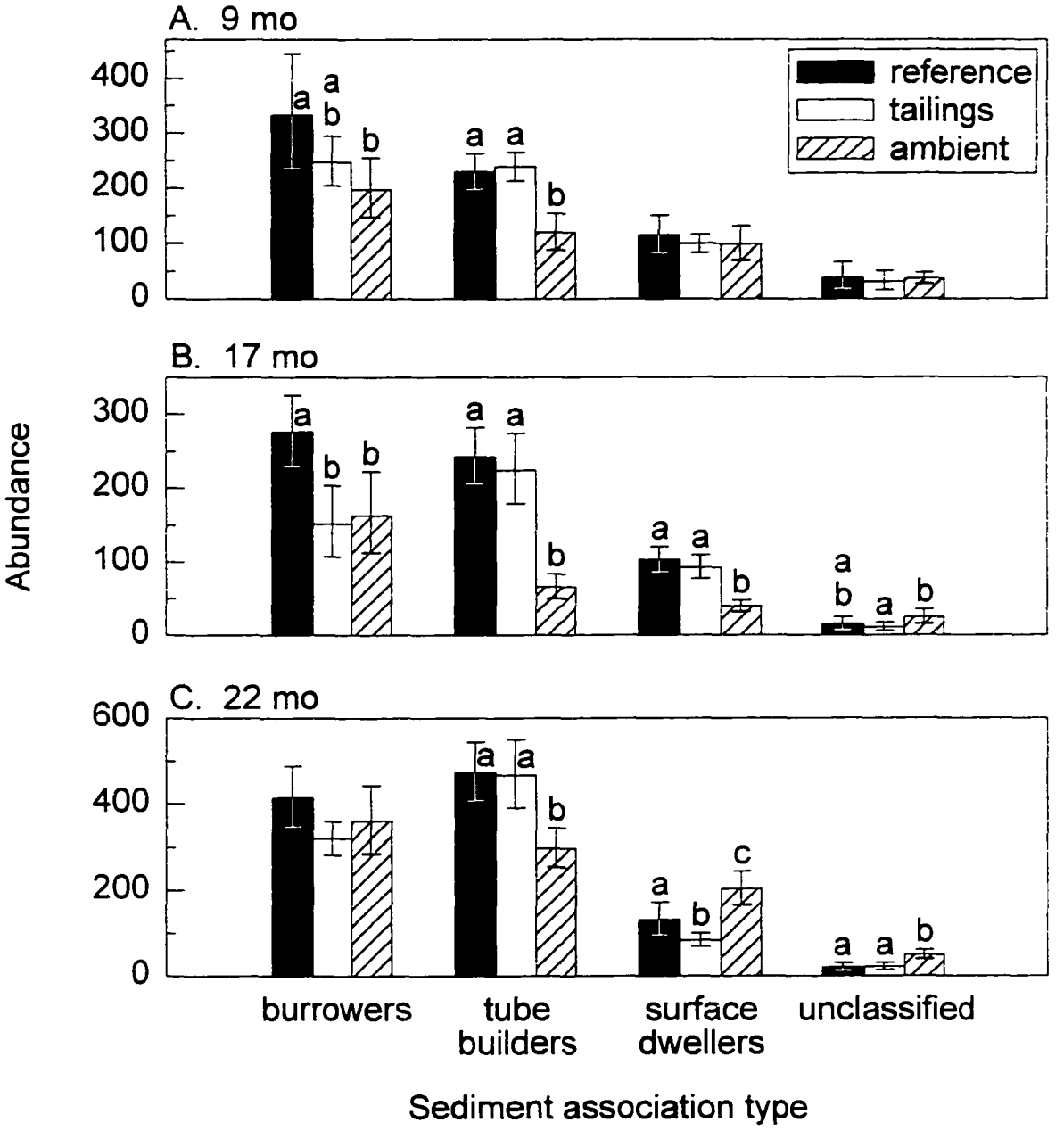

Figure 13. Abundance by sediment association type of macrofauna from the colonization experiment. The 9, 17, and 22 month colonization periods were sampling periods 1, 2, and 3, respectively. Bars represent means and 95% confidence intervals for entire samples (177 cm^2). Groups of bars for each sediment association type within a sampling period that have letters but do not share a letter were significantly different (Tukey's, $p \leq 0.05$).

dwellers reflected the abundance of *N. cornuta*. For each sampling period, there were significantly fewer tube builders in ambient sediment than in reference sediment and tailings, due mainly to lower abundance of *G. oculata* in ambient sediment. Minimum detectable differences for functional groups as a percent of the reference mean ranged from 26 to 151 ($\alpha = 0.05$, $1-\beta = 0.8$).

Macrofauna at a candidate STD site

All of the candidate STD site samples from Lynn Canal (Figure 1) were finer grained than the reference sediment and tailings, based on the observation that nearly 100% passed through a 250 μm sieve. The samples from the shallow transect, which were at the base of a steep slope, were slightly coarser than the deep samples and contained some woody debris.

Most of the taxa that were found in both the Auke Bay and Lynn Canal samples were rare in the Lynn Canal samples (Table 4), with the exception of *G. oculata*. Taxonomic overlap was similar for reference sediment and tailings when compared to the Lynn Canal samples. A higher percentage of the shallow taxa than the deep taxa were common to the Auke Bay samples. Of the taxa that were in the shallow Lynn Canal samples, only *M. californiensis*, *G. oculata*, and *Lysippe* sp., all polychaetes, and *Axiopsida serricata*, a bivalve, were present in reference sediment or tailings at an overall average abundance of $\geq$ 10 organisms/tray. Of the deep Lynn Canal taxa, only *A. serricata* exceeded an average of $\geq$ 10 organisms/tray in the Auke Bay samples.

Table 4. Comparison of macrofauna from the candidate STD site in Lynn Canal to samples from the colonization experiment in Auke Bay. All data were pooled across replicates and sampling periods. Average depth of samples: shallow Lynn Canal, 187 m; deep Lynn Canal, 306 m; Auke Bay, 21 m.

Comparison	Reference sediment	Tailings	Ambient sediment
% of shallow Lynn Canal taxa common to Auke Bay samples	36.1	38.9	44.4
% of deep Lynn Canal taxa common to Auke Bay samples	26.1	26.1	17.4
Abundance of shallow Lynn Canal taxa common to Auke Bay samples as % of total shallow Lynn Canal abundance	20.3	22.0	22.0
Abundance of deep Lynn Canal taxa common to Auke Bay samples as % of total deep Lynn Canal abundance	7.3	7.8	6.1

The deep samples averaged 30.0 individuals and 8.8 taxa/177 cm^2 sample. The shallow samples averaged 80.0 individuals and 12.4 taxa. A total of 23 and 36 taxa were identified in the deep and shallow samples, respectively. Eight of these taxa were exclusive to the deep samples and 21 were exclusive to the shallow.

The 3 most abundant taxa for both transects were polychaetes. Starting with the most abundant, these taxa were *Cossura* sp., *Nephtys* cf. *cornuta*, and *Levinsenia gracilis* for the deep transect. For the shallow transect, the most abundant taxa were *L. gracilis*, *N.* cf. *cornuta*, and *Cossura* sp. These taxa comprised 65 and 63% of the total abundance for the deep and shallow transects, respectively. *Cossura* sp. is a surface deposit feeder (Blake 1993). *L. gracilis* is a burrowing, surface deposit feeder (Fauchald and Jumars 1979, Gaston *et al.* 1992). *N. cornuta* is a burrowing carnivore (Fauchald and Jumars 1979). Overall, the Lynn Canal samples were comprised mainly of burrowers, surface deposit feeders, and carnivores.

Discussion

Assemblage comparisons

This study was designed to assess the ability of macrofauna to recolonize tailings after obliteration by STD. Macrofauna that colonized reference sediment were assumed to be representative of a soft-bottom assemblage colonizing natural marine sediment under the experimental conditions. Any effects that the experimental conditions (e.g., tray effects) had on macrofauna were assumed to be equal for

reference sediment and tailings, isolating sediment type as the cause of potential differences in macrofauna. It was also assumed that tray effects did not overwhelm sediment effects.

Despite a few statistically significant differences between the macrofaunal assemblages in reference sediment and tailings, the assemblages as a whole were similar. Most of the data were analyzed by abundance rather than biomass since abundance data were less variable. This approach emphasized the differences between reference sediment and tailings since total biomass in reference sediment and tailings was more similar than total abundance (Figure 6). In addition, since the ANOVA comparisons were done within sampling periods, the chance of detecting differences across periods due to chance alone was not controlled for. That is, the type I error rate of all comparisons taken together was greater than $\alpha = 0.05$. Hence, some of the differences deemed significant at $p \leq 0.05$, when considered together with other ANOVA comparisons, may not have been significant.

Correspondence analysis was the most comprehensive test of the similarity of the reference sediment and tailings assemblages (Figure 10). The inability to distinguish between reference sediment and tailings assemblages using correspondence analysis indicated that differences in abundance that were detected using ANOVA (Figures 6, 11-13) were outweighed by the overall similarity of the assemblages. In contrast, the ambient assemblage was distinguishable from reference sediment and tailings assemblages using correspondence analysis and

ANOVA.

Most of the differences between the reference sediment and tailings assemblages were due mainly to reduced abundance of *M. californiensis* in tailings. *M. californiensis* is a capitellid, described as a burrowing, subsurface deposit feeder (Barnes 1987, Kalke and Montagna 1991, Lastra *et al.* 1991). *M. californiensis* has been classified as an r-strategist (Santos and Simon 1980b) and their larvae are planktotrophic (Levin 1984). These characteristics suggest extensive larval dispersal and the ability to colonize large, disturbed areas such as an STD site. *M. californiensis* was rare at the candidate STD site in Lynn Canal (3 individuals were found in 11 samples). However, *M. californiensis* might be more abundant during the early stages of recolonization at this site if STD were employed.

The smaller size (biomass/abundance) of *M. californiensis* in tailings compared to reference sediment indicated a growth reduction or a younger age distribution (Figure 11). There were no significant reductions in the size of other abundant taxa in tailings. *Prionospio steenstrupi*, a tube building, surface deposit feeder, was significantly larger in tailings than in reference sediment. Since growth can be a sensitive indicator of stress (Paffenhofer 1972, Thain 1984), the overall lack of differences in biomass/abundance of abundant taxa in tailings and reference sediment indicated a lack of effects at the organism level.

The most common functional groups in reference sediment were also abundant in tailings (Figures 12 and 13). This sharing of

functional groups suggested that the functions performed by macrofauna in the reference sediment were also being performed in the tailings, although to somewhat varying degrees. These functions may have included near-surface taxa serving as prey for demersal predators, retention of organic matter by tube builders, and sediment irrigation by subsurface deposit feeders (Rhoads and Germano 1986).

Sediment differences

The lack of differences in reference sediment and tailings assemblages indicated that physical and chemical differences between the two sediment types were inconsequential. Among the sediment characteristics that could have influenced the assemblages were grain size, metal content, residual milling reagents, organic content, and compaction.

The reference sediment was sieved to remove large particles since effects on macrofauna resulting from differences in grain size between reference sediment and tailings were not of interest. A subtidal sediment tray experiment (Zajac and Whitlatch 1982a) corroborated the apparent unimportance of grain size differences that remained between reference sediment and tailings after sieving (Figure 5). The study was conducted in Connecticut, USA (41° 21'N) at a maximum water depth of 1 m. Few differences were found in assemblages of macrofauna colonizing sediment comprised of 10% versus 80% silt-clay content.

Another sediment characteristic that could have influenced the

assemblages was metal content. STD can lead to metal contamination in host waters if tailings are reactive in seawater (Johansen *et al.* 1991). To assess this risk, a study was conducted (Titan Environmental Corporation 1996) in conjunction with the current colonization experiment. The study addressed metal release using columns of the same reference sediment and tailings at the same site as the colonization experiment. The columns were retrieved over a 17 mo period and analyzed for 6 trace metals. Pore water concentrations of Cd, Cu, Pb, and Zn were similar in reference sediment and tailings. The concentration of Fe was slightly higher and the concentration of Mn was 4 to 5x lower in tailings pore water, relative to reference sediment. Differences in release of metals from the sediments were not sufficient to cause differences in the macrofaunal assemblages.

A unique odor was easily detected from the tailings after milling, presumably due to the presence of milling reagents. This odor was still detectable when sieving the tailings samples that were collected after 9 mo *in situ*, although it was not detected while sieving the 17 and 22 mo samples. Due to the lack of differences in reference sediment and tailings assemblages, milling reagents that may have remained associated with the tailings were present at non-toxic concentrations.

The most apparent differences between reference sediment and tailings were relatively low organic content and high compaction of tailings. These factors may have been the cause of reduced abundance of subsurface deposit feeders (Figure 12) and burrowers (Figure 13)

since organic content and compaction should be most likely to impact subsurface taxa. However, tube builders were equally abundant in tailings and were found throughout the depth of the tailings trays, indicating that compaction did not hinder all subsurface taxa. Since subsurface taxa were able to colonize the tailings, bioturbation may have decreased compaction over the course of the experiment. Also, bioturbation, combined with the addition of natural, settled sediment, may have increased the organic content of the tailings. Therefore, the most likely time for high compaction and low organic content of tailings to have inhibited colonization by macrofauna should have been during sampling period 1.

Ambient assemblage

Recovery of benthic assemblages in sediment trays is commonly assessed through comparison to the ambient assemblage (Levin and Smith 1984, Grassle and Morse-Porteous 1987). For most attributes, the ambient assemblage was significantly different from the tray assemblages. The ambient assemblage was not used as a reference since, due to tray effects, the tray assemblages may never have become indistinguishable from the ambient assemblage. If the tray assemblages had become indistinguishable from the ambient assemblage, tray effects could have been ruled out and it could have been interpreted as evidence that the tray assemblages had reached the same successional stage as the ambient sediment.

A study on the effects of patch size and substrate isolation in

an intertidal zone demonstrated that there was no clear effect on larval recruitment in containers of sediment ranging from 50 to 1,750 cm^2 (Smith and Brumsickle 1989). However, the same study found major differences in recruitment when the container edge was raised 5 cm above the ambient sediment and surrounded by a plate so that the diagonal distance to ambient sediment was 9 cm. These authors concluded that colonization trays are flawed mimics of the natural sea floor, citing exclusion of surrounding benthos or anomalous flow conditions as possible causes.

For the current study, the inner trays were 177 cm^2 (Figure 3). They were isolated from the ambient sediment 10 cm vertically and 6.5 cm horizontally due to the outer tray. These dimensions should have been sufficient to significantly reduce immigration by ambient juveniles and adults without impeding larval settlement (Smith and Brumsickle 1989, Diaz-Castañeda *et al.* 1993). These tray effects were desirable since the objective was not to mimic the natural sea floor, but to mimic recolonization of a defaunated sea floor. STD can produce tailings deposits on the order of square kilometers, with near defaunation in areas receiving the heaviest deposition (Ellis *et al.* 1995a). Recolonization of large, defaunated areas is mainly through dispersal and settlement of meroplankton (Santos and Simon 1980a, Taylor 1986).

Other tray effects, such as effects on larval settlement resulting from flow anomalies (Butman 1987, Snelgrove 1994), were equal for reference sediment and tailings. Therefore, tray effects

did not interfere with the comparison of recruitment success of macrofauna. However, flow anomalies remain a possible source of bias between the ambient and tray assemblages since effects of the outer trays were not assessed.

A possible source of bias among ambient sediment, reference sediment, and tailings was the relative ease in which settling macrofauna may have avoided the small area of the tray sediments. Since few differences were found between the reference sediment and tailings assemblages, avoidance was either insignificant or similar for both sediment types. Regardless, avoidance did not appear to have been a source of bias in comparing reference sediment and tailings, but may have biased comparison of the tray and ambient assemblages.

The outer trays of reference sediment were intended to detect avoidance behavior. *M. californiensis* was the only candidate taxon to test the method since it was the only taxon that was abundant in reference sediment and consistently less abundant in tailings. Higher densities of *M. californiensis* in the outer tailings trays would have indicated that avoidance contributed to reduced *M. californiensis* abundance in tailings. However, the labor involved to process the outer tray sediment was not deemed worthwhile since there was only one candidate taxon. Had there been more candidate taxa, the chances of detecting avoidance behavior would have been greater.

The trays may also have excluded large predators. To simulate a recently deposited expanse of tailings, exclusion of large, less mobile predators such as hermit crabs and starfish may be realistic.

Exclusion of highly mobile predators, such as flatfish, anomuran and brachyuran crabs, and shrimp may be less realistic. Data on predator exclusion were not collected. Any effect that predator exclusion may have had was equal for reference sediment and tailings. A predator exclusion experiment demonstrated that reduced predation by crabs and fish can result in increased density and diversity of macrofauna (Virnstein 1977). This suggests that predator exclusion may have been a factor in the differences between ambient and tray assemblages since the tray assemblages were generally more dense and taxonomically rich (Figures 6 and 9).

The finding that the numbers of taxa and taxa richness using rarefaction methodology were greater in the trays than in the ambient sediment (Figures 6 and 9) could be interpreted as reflecting an initial, non-interactive stage of succession. As the density of animals increases, competitive interactions can lead to a reduction in the numbers of taxa and diversity (Thistle 1981, Hughes 1984). This scenario, however, is inconsistent with the current findings since the trays contained higher densities of macrofauna than did ambient sediment, in addition to greater taxa richness.

Species level analysis can provide evidence of assemblage maturity since early successional stage assemblages are often characterized by opportunistic species. Zajac and Whitlatch (1982a) defined an opportunistic species as one that displays a significantly increased population size above ambient levels following a disturbance. By this definition, *M. californiensis* and *G. oculata*,

the most abundant taxa in the trays, were opportunistic. Capitellids (*M. californiensis*) and spionids (*P. steenstrupi*) are cited as early successional taxa, as are small tube building polychaetes located near the surface (*G. oculata*) (Rhoads and Germano 1986). However, these same taxa were also most abundant in ambient sediment, suggesting that they are capable of successfully competing in a mature community and therefore not strictly opportunistic, or that the ambient sediment was at an early successional stage. The Auke Bay study site may be subject to constant, natural perturbations that maintain the ambient assemblage at an early, successional stage.

In short, the successional stage of the tray assemblages was not revealed by comparison with the ambient assemblage. It is possible that the reference and tailings assemblages would have diverged as they matured, if they had in fact not reached an equilibrium after 22 mo. While this could only have been determined through a longer experiment, the influence of sediment type on the assemblages probably decreased over time. As discussed above, bioturbation and settlement of natural sediment probably decreased differences between reference sediment and tailings. Consequently, the greatest chance of detecting differences in the reference sediment and tailings assemblages probably occurred during sampling period 1.

To assess tray effects, one alternative would have been to use unsieved reference sediment. This may have increased the chances of the reference sediment assemblage attaining equilibrium with the ambient assemblage. However, equilibrium between the reference

sediment and ambient assemblages still may not have occurred due to disruption of sorting and biogenic structure and other reasons associated with collecting the sediment, in addition to tray effects. Use of unsieved reference sediment would have also increased the possibility of differences in tailings and reference sediment assemblages resulting from differences in grain size. Since tailings and sediment from the candidate STD site were fine grained, effects of large particles were not of interest.

Extrapolation of results

Ideally, a case study would be conducted with sediment collected from a proposed tailings depositional area since the sediment at every STD site, and within a site, will differ. The reference sediment used for this study was not identical to the sediment at the candidate STD site in Lynn Canal, however, this was not crucial. Comparison of colonization in tailings to any naturally occurring, uncontaminated, near-shore, fine-grained, marine sediment would have provided perspective. Tailings are also site-specific and they can vary in composition over the life of a mine due to variations in ore bodies and milling procedures. Kensington tailings were selected for this study because they were available and because the Kensington mine was previously identified as a candidate for STD (Coldwell and Gensler 1993).

The methods that were used for this study were designed to be applicable to any proposed STD operation, or other activities such as

dredge spoil disposal. An *in situ* design was employed to minimize assumptions that are problematic in laboratory to field extrapolations. Single species laboratory experiments rely upon numerous assumptions for extrapolation beyond the chosen species and beyond the laboratory conditions. The *in situ* design precluded prediction of the actual rate of recovery after STD due to tray, location, and depth effects. However, the methods were an improvement over laboratory experiments. A study such as this could be conducted at a proposed STD site if a submersible were used, reducing assumptions to only those related to tray effects.

For the most part, taxa that were found at both the Auke Bay study site and the Lynn Canal candidate STD site were not abundant at either site (Table 4). Despite these site differences, the results from the colonization study can be extrapolated to the Lynn Canal site. It is unlikely that the Auke Bay assemblage was unique in its lack of response to the tailings compared to the reference sediment. The chances that the results of this experiment were specific to the study site decreased as the number of taxa colonizing both the reference sediment and tailings increased.

The greatest drawback to the study site location was depth. Benthos recolonize at a slower rate with increasing depth (Grassle 1977, Levin and Smith 1984, Smith and Hessler 1987). Unfortunately, most of the comparative data are from the intertidal zone, very shallow subtidal, or the deep sea (Thistle 1981). There is little information on benthic colonization at the depth that this experiment

was conducted at or the typical depths of past and potential STD sites along the coasts of British Columbia and Alaska (Coldwell and Gensler 1993). Rates of recolonization in the deep sea can range from days to years, depending on the species, although abundant species often recover over a period of months (Kukert and Smith 1992). Given the finding of similar assemblages in reference sediment and tailings, it may be inferred that the tailings would not significantly reduce the rate of recolonization compared to the rate in the reference sediment. However, the actual rate could not be determined and would be limited by local recruitment processes.

Recolonization at STD sites

Studies on benthic recolonization at actual STD sites are scarce. The Island Copper Mine on Vancouver Island, British Columbia, Canada, may have the most extensive data base (Ellis *et al.* 1995a). This operation did not cease until 1996 and post-operation data were not available at the time of this writing. After 22 yr of STD, 3 yr prior to closure, the tailings deposit extended approximately 16 km along the axial length of a fjord and covered the majority of its 1.8 km width (Poling *et al.* 1993).

Benthic monitoring indicated various levels of impact, reducing with distance from the outfall (Ellis *et al.* 1995a). Samples collected nearest the outfall generally contained 1 to 5 species of macrofauna. Assessment of the rate of recovery was complicated by varying rates of tailings deposition at and among the sampling

locations. The rate of tailings deposition was suspected to be the primary determinant of benthic impact, as opposed to the accumulated depth of tailings, water depth, or sediment metal concentrations.

Taylor (1986) conducted a comparison of colonization by macrofauna in tailings from the Island Copper Mine and in marble sand. The 2 sediment types were colonized by similar taxonomic assemblages, although tailings retarded the rate of community development. Since the 2 sediment types differed primarily in chemical composition, some chemical component of the tailings may have reduced the rate of community development in the tailings (Taylor 1986, Ellis and Taylor 1988).

The Kitsault Mine, located on the coast of British Columbia, employed STD for 18 mo. Benthic samples collected during and after the operation revealed some recovery 11 mo after the operation ceased (Kathman *et al.* 1983, Kathman *et al.* 1984, Brinkhurst *et al.* 1987). Recovery in terms of abundance and number of taxa was complete 4 yr after the operation, although differences in the proportional representations of species were still detected (Brinkhurst *et al.* 1987). The conclusions pertaining to the Kitsault operation should be viewed with caution due to reliance upon, and low replication of reference areas (see Underwood 1994).

Similarly, assignment of cause and effect was hindered by pseudoreplication in a study at another site on the coast of British Columbia (Ellis and Hoover 1990b). The site received tailings via riverine discharge for 75 yr. Differences between the benthic

assemblages at a reference site and the tailings site were detected 12 yr after the tailings discharge ceased. Without interspersed replicates of tailings and reference sites or extensive pre-impact data (see Stewart-Oaten *et al.* 1986), the detected differences could not be justifiably attributed to the presence of tailings.

Conclusions

This *in situ* experiment was an effective means to assess the ability of macrofauna to recolonize an area that has been obliterated by STD. The methods were not designed to assess the actual rate of recovery and the results did not allow conclusions to be drawn pertaining to reestablishment of a pre-impact assemblage. Nonetheless, recolonization by macrofauna after STD should not be inhibited by the tailings used in this study. Recolonization should be substantially similar to that in barren, natural sediment and controlled by natural recruitment processes.

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Literature Cited

American Society for Testing and Materials. 1990. Test method for particle size analysis of soils. *Annual Book of ASTM Standards*, designation: D 422-63. ASTM, Philadelphia, PA, USA.

Arntz, W.E. 1978. The "upper part" of the benthic food web: the role of macrobenthos in the western Baltic. *Rapp. P.-v. Reun. Cons. int. Explor. Mer.* 173:85-100.

Barnes, R.D. 1987. *Invertebrate Zoology*. 5th ed., Saunders College Publishing, Philadelphia, PA, USA. 893 pp.

Blake, J.A. 1993. Life history analysis of five dominant infaunal polychaete species from the continental slope off North Carolina. *J. Mar. Biol. Assoc. U.K.* 73:123-141.

Bonsdorff, E. and C. Österman. 1985. The establishment, succession and dynamics of a zoobenthic community - an experimental study. *Proceedings*, 19th European Biology Symposium, Plymouth, Devon, UK, September 16-21, 1984, Cambridge University Press, New York, NY, USA, pp. 287-297.

Brinkhurst, R.O., B.J. Burd, and R.D. Kathman. 1987. Benthic studies in Alice Arm B.C., following cessation of mine tailings disposal, 1982 to 1986. *Can. Tech. Rep. Hydrog. Ocean Sci.* No. 89, 45 pp.

Buchanan, J.B. 1984. Sediment analysis. In: *Methods for the Study of Marine Benthos*. 2nd ed., N.A. Holme and A.D. McIntyre, eds., Blackwell Scientific Publications, Oxford, England, pp. 41-65.

Butman, C.A. 1987. Larval settlement of soft-sediment invertebrates: the spatial scales of pattern explained by active habitat selection and the emerging role of hydrodynamical processes. *Oceanogr. Mar. Biol. Annu. Rev.* 25:113-165.

Caldwell, J.A. and J.D. Welsh. 1982. Tailings disposal in rugged, high precipitation environments: an overview and comparative assessment. *Marine Tailings Disposal*, D.V. Ellis, ed., Ann Arbor Science Publishers, Ann Arbor, MI, USA, pp. 5-62.

Coldwell, J.R. and E.C. Gensler. 1993. Potential for submarine tailings disposal to affect the availability of minerals from United States coastal areas. U.S. Bureau of Mines, OFR 101-93, 49 pp.

Coyle, K.O. and A.J. Paul. 1990. Interannual variations in zooplankton population and biomass during the spring bloom in an Alaskan subarctic embayment. *APPRISE - Interannual Variability and Fisheries Recruitment,* D.A. Ziemann and K.W. Fulton-Bennett, eds., The Oceanic Institute, Honolulu, HI, USA, pp. 179-228.

Coyle, K.O. and T.C. Shirley. 1990. A review of fisheries and oceanographic research in Auke Bay, Alaska and vicinity, 1966-1985. *APPRISE - Interannual Variability and Fisheries Recruitment,* D.A. Ziemann and K.W. Fulton-Bennett, eds., The Oceanic Institute, Honolulu, HI, USA, pp. 1-73.

Crisp, D.J. 1971. Energy flow measurements. In: *Methods for the Study of Marine Benthos,* IBP Handbook No. 16, N.A. Holme and A.D. McIntyre, eds., Blackwell Scientific Publications, Oxford, England, pp. 263-265.

Diaz-Castañeda, V., S. Frontier, and V. Arenas. 1993. Experimental re-establishment of a soft bottom community: utilization of multivariate analyses to characterize different benthic recruitments. *Estuarine Coastal Shelf Sci.* 37:387-402.

Ellis, D.V. and P.M. Hoover. 1990a. Benthos on tailings beds from an abandoned coastal mine. *Mar. Pollut. Bull.* 21:477-480.

Ellis, D.V. and P.M. Hoover. 1990b. Benthos recolonizing mine tailings in British Columbia fjords. *Mar. Mining* 9:441-457.

Ellis, D.V. and L.A. Taylor. 1988. Biological engineering of marine tailings beds. *Environmental Management of Solid Waste,* Springer-Verlag, Berlin, Germany, pp. 185-207.

Ellis, D., G. Poling, and C. Pelletier. 1994. *Case Studies of Submarine Tailings Disposal: Volume II - Worldwide Case Histories and Screening Criteria.* U.S. Bureau of Mines, OFR 37-94, 125 pp.

Ellis, D.V., T.F. Pedersen, G.W. Poling, C. Pelletier, and I. Horne. 1995a. Review of 23 years of STD: Island Copper Mine, Canada. *Mar. Geores. Geotech.* 13:59-99.

Ellis, D.V., G.W. Poling, and R.L. Baer. 1995b. Submarine tailings disposal (STD) for mines: an introduction. *Mar. Geores. Geotech.* 13:3-18.

Fauchald, K. 1977. *The Polychaete Worms.* Natural History Museum of Los Angeles, Los Angeles, CA, USA. 188 pp.

Fauchald, K. and P.A. Jumars. 1979. The diet of worms: a study of polychaete feeding guilds. *Oceanogr. Mar. Biol. Ann. Rev.* 17:193-284.

Gaston, G.R., J.A. McLelland, and R.W. Heard. 1992. Feeding biology, distribution, and ecology of two species of benthic polychaetes: *Paracnis fulgens* and *Paraonis pygoenigmatica* (Polychaeta: Paraonidae). *Gulf Res. Rep.* 8:395-400.

Grassle, J.F. 1977. Slow recolonisation of deep-sea sediment. *Nature* 265:618-619.

Grassle, J.F. and L.S. Morse-Porteous. 1987. Macrofaunal colonization of disturbed deep-sea environments and the structure of deep-sea benthic communities. *Deep Sea Res.* 34:1911-1950.

Grassle, J.P. and J.F. Grassle. 1984. The utility of studying the effects of pollutants on single species populations in benthos of mesocosms and coastal ecosystems. *Concepts in Marine Pollution Measurements*. H.H. White, ed., Maryland Sea Grant College, College Park, MD, USA, pp. 621-642.

Greenacre, M.J. and L.G. Underhill. 1982. Scaling a data matrix in a low-dimensional Euclidean Space. *Topics in Applied Multivariate Analysis*. D.M. Hawkins, ed., Cambridge University Press, Cambridge, England, pp. 183-266.

Greenacre, M.J. and E.S. Vrba. 1984. Graphical display and interpretation of antelope census data in African wildlife areas, using correspondence analysis. *Ecology* 65:984-997.

Hay, A.E., J.W. Murray, and R.W. Burling. 1983. Submarine channels in Rupert Inlet, British Columbia: II. sediments and sedimentary structures. *Sed. Geol.* 36:317-339.

Hesse, C.A. and D.V. Ellis. 1995. Quartz Hill, Alaska: a case history of engineering and environmental requirements for STD in the USA. *Mar. Geores. Geotech.* 13:135-182.

Hughes, R.G. 1984. A model of the structure and dynamics of benthic marine invertebrate communities. *Mar. Ecol. Prog. Ser.* 15:1-11.

Johansen, P., M.M. Hansen, G. Asmund, and P.B. Nielsen. 1991. Marine organisms as indicators of heavy metal pollution - experience from 16 years of monitoring at a lead zinc mine in Greenland. *Chem. Ecol.* 5:35-55.

Jones, S.G. and D.V. Ellis. 1995. Deep water STD at the Misima gold and silver mine, Papua, New Guinea. *Mar. Geores. Geotech.* 13:183-200.

Kalke, R.D. and P.A. Montagna. 1991. The effect of freshwater inflow on macrobenthos in the Lavaca River delta and upper Lavaca Bay, Texas. *Contrib. Mar. Sci.* 32:49-71.

Karinen, J.F. 1985. Distribution of heavy metals and hydrocarbons in Auke Bay sediments and in biota near active marinas. *Final Environmental Impact Statement, Auke Bay Breakwater and Related Marina Developments*, U.S. Army Corps of Engineers, 46 pp.

Kathman, R.D., R.O. Brinkhurst, R.E. Woods, and D.C. Jeffries. 1983. Benthic studies in Alice Arm and Hastings Arm, B.C., in relation to mine tailings dispersal. *Can. Tech. Rep. Hydrog. Ocean Sci.* 22, 30 pp.

Kathman, R.D., R.O. Brinkhurst, R.E. Woods, and S.F. Cross. 1984. Benthic studies in Alice Arm, B.C., following cessation of mine tailings disposal. *Can. Tech. Rep. Hydrog. Ocean Sci.* 37, 57 pp.

Kozloff, E.N. 1987. *Marine Invertebrates of the Pacific Northwest* University of Washington Press, Seattle, WA, USA, 511 pp.

Kukert, H. and C.R. Smith. 1992. Disturbance, colonization and succession in a deep-sea sediment community: artificial-mound experiments. *Deep Sea Res.* 39:1349-1371.

Lastra, M., J.M. Parada, A. Sanchez, and J. Mora. 1991. Temporal evolution of an *Abra abra* community affected by harbor dredging activities in Santander Bay (northern Spain). *Thalassas* 9:87-97.

Leduc, G., H. Ruber, and M. Webb. 1975. Toxicity studies in relation to mining in the Northwest Territories. Department of Indian Affairs and Northern Development, Ottawa, Canada, ALUR 73-74-34, 16 pp.

Levin, L.A. 1984. Life history and dispersal patterns in a dense infaunal polychaete assemblage: community structure and response to disturbance. *Ecology* 65:1185-1200.

Levin, L.A. and C.R. Smith. 1984. Response of background fauna to disturbance and enrichment in the deep sea: a sediment tray experiment. *Deep Sea Res.* 31:1277-1285.

Paffenhofer, G.A. 1972. The effects of suspended 'red mud' on mortality, body weight, and growth of the marine planktonic copepod, *Calanus helgolandicus*. *Water Air Soil Pollut.* 1:314-321.

Pedersen, T.F. and A.J. Losher. 1988. Diagenetic processes in aquatic mine tailings deposits in British Columbia. *Chemistry and Biology of Solid Waste: Dredged Material and Mine Tailings*, W. Salomons and U. Förstner, eds., Springer-Verlag, Berlin, Germany. pp. 238-258.

Poling, G.W. 1982. Characteristics of mill tailings and their behavior in marine environments. *Marine Tailings Disposal*. D.V. Ellis, ed., Ann Arbor Science Publishers, Ann Arbor, MI, USA, pp. 63-84.

Poling, G.W., D.V. Ellis, C.A. Pelletier, T.F. Pedersen, and C.A. Hesse. 1993. Case studies of submarine tailings disposal: volume I - North American examples. U.S. Bureau of Mines, OFR 89-93.

Rhoads, D.C. and J.D. Germano. 1986. Interpreting long-term changes in benthic community structure: a new protocol. *Hydrobiol.* 142:291-308.

Santa Barbara Museum of Natural History. 1996. *Taxonomic Atlas of the Benthic Fauna of the Santa Maria Basin and the Western Santa Barbara Channel, Volume 6, The Annelida Part 3 - Polychaeta: Orbiniidae to Cossuridae*, J.A. Blake, B. Hilbig and P.H. Scott, eds., Santa Barbara, CA, USA, 418 pp.

Santos, S.L. and J.L. Simon. 1980a. Marine soft-bottom community establishment following annual defaunation: larval or adult recruitment?. *Mar. Ecol. Prog. Ser.* 2:235-241.

Santos, S.L. and J.L. Simon. 1980b. Response of soft-bottom benthos to annual catastrophic disturbance in a south Florida estuary. *Mar. Ecol. Prog. Ser.* 3:347-355.

Short, J.W., S.D. Rice, C.C. Brodersen, and W.B. Stickle. 1989. Occurence of tri-*n*-butyltin-caused imposex in the North Pacific marine snail *Nucella lima* in Auke Bay, Alaska. *Mar. Biol.* 102:291-297.

Smith, C.R. and S.J. Brumsickle. 1989. The effects of patch size and substrate isolation on colonization modes and rates in intertidal sediment. *Limnol. Oceanogr.* 34:1263-1277.

Smith, C.R. and R.R. Hessler. 1987. Colonization and succession in deep-sea ecosystems. *Trends Ecol. Evolut.* 2:359-363.

Smith, W. and J.F. Grassle. 1977. Sampling properties of a family of diversity measures. *Biom.* 33:283-292.

Snelgrove, P.V.R. 1994. Hydrodynamic enhancement of invertebrate larval settlement in microdepositional environments: colonization tray experiments in a muddy habitat. *J. Exp. Mar. Biol. Ecol.* 176:149-166.

SPSS, Inc. 1997. SYSTAT 7.0. Chicago, IL, USA.

Stewart-Oaten, A.S., W.W. Murdoch, and K.R. Parker. 1986. Environmental impact assessment: "pseudoreplication" in time?. *Ecology* 67:929-940.

Taylor, L.A. 1986. Marine macrobenthic colonization of mine tailings in Rupert Inlet, British Columbia. M.S. thesis, University of Victoria, 155 pp.

Thain, J.E. 1984. Effects of mercury on the prosobranch mollusc *Crepidula fornicata*: acute lethal toxicity and effects on growth and reproduction of chronic exposure. *Mar. Environ. Res.* 12:285-309.

Thistle, D. 1981. Natural physical disturbances and communities of marine soft bottoms. *Mar. Ecol. Prog. Ser.* 6:223-228.

Titan Environmental Corporation. 1996. Chemical release and recolonization characteristics of tailings deposited in marine waters, Part I. Prepared for Echo Bay Mines, Juneau, AK, USA.

Twenhofel, W.H. and S.A. Tyler. 1941. *Methods of Study of Sediments* McGraw-Hill Book Co., New York, NY, USA, 183 pp.

Underwood, A.J. 1994. On beyond BACI: sampling designs that might reliably detect environmental disturbances. *Ecol. App.* 4:3-15.

Virnstein, R.W. 1977. The importance of predation by crabs and fishes on benthic infauna in Chesapeake Bay. *Ecology* 58:1199-1217.

Zajac, R.N. and R.B. Whitlatch. 1982a. Responses of estuarine infauna to disturbance. I. Spatial and temporal variation of initial recolonization. *Mar. Ecol. Prog. Ser.* 10:1-14.

Zajac, R.N. and R.B. Whitlatch. 1982b. Responses of estuarine infauna to disturbance. II. Spatial and temporal variation of succession. *Mar. Ecol. Prog. Ser.* 10:15-27.

Zar, J.H. 1996. *Biostatistical Analysis* 3rd ed., Prentice-Hall, Upper Saddle River, NJ, USA, 662 pp.

Ziemann, D.A. and K.W. Fulton-Bennett (eds). 1990. APPRISE-Interannual Variability and Fisheries Recruitment. The Oceanic Institute. Honolulu, HI, USA. 400 pp.

Chapter 3

Toxicity of Gold Mill Effluent to Marine Fish and Crustaceans: An Example of Response-Dependence in Comparison of Species Sensitivity

Abstract

The acute toxicity of gold mill effluent was compared for two reference species: juvenile mysid shrimp (*Mysidopsis bahia*); larval sheepshead minnows (*Cyprinodon variegatus*), and larvae of three species that are indigenous to the southern coast of Alaska: red king crab (*Paralithodes camtschaticus*); northern shrimp (*Pandalus borealis*); Pacific herring (*Clupea harengus pallasi*). Concentration-response relationships for the effluent, adjusted to equal the osmolality and pH of seawater, were determined over 24 h for immobility, paralysis, and death of the species. For all responses, mysid shrimp and sheepshead minnows were the most and least sensitive species, respectively. However, the magnitude of differences and rank of sensitivity of the indigenous species depended on the type of response that was used for the comparison. As such, an overall ranking of species sensitivity could not be made, demonstrating that a single response, including lethality, can be insufficient when comparing the acute sensitivity of species to a toxicant. When transferred to seawater, northern shrimp regained mobility after being paralyzed by effluent. Mysid shrimp and Pacific herring did not regain mobility, demonstrating the need to understand the biological

relevance of sublethal responses. For the life stages and species included in this study, it is unlikely that exposure to this gold mill effluent in the ocean could be sufficient to cause acute toxicity.

Introduction

Coastal metal mining operations may discharge water used for ore processing to near-shore, marine waters. Some mills are able to recirculate and re-use most or all process water (Read and Manser 1976). Others may use process water to carry tailings to a discharge point for marine disposal (Ellis *et al.* 1995, Poling and Ellis 1995). In the U.S., discharge of ore processing water is prohibited, but there is an allowance for spillover from a tailings impoundment if net, annual precipitation at a mill site exceeds the capacity of a mill to re-use impounded water. Since the location of an ore processing mill is often dictated by the location of an ore body, mills may be operated in areas deemed pristine or environmentally sensitive, including current and potential projects along the coast of southern Alaska (Coldwell and Gensler 1993).

Marine biota may be exposed to ore processing water near a marine discharge. To monitor the risk of toxicity, whole effluent toxicity (WET) tests are often required (e.g. U.S. Environmental Protection Agency 1993). Early life stage mysid shrimp (*Mysidopsis bahia*), an estuarine crustacean, and sheepshead minnows, (*Cyprinodon variegatus*), an estuarine fish, are among the commonly used species for WET testing. Early life stages are often the most sensitive to toxicants (McKim 1977, Woltering 1984). Use of indigenous species for WET tests can be useful for assessing site-specific risks of toxicity. However, use of indigenous species requires that they be cultured and tested as needed and be equally or more sensitive to effluent than

reference species (U.S. Environmental Protection Agency 1993). These constraints may preclude use of ecologically or economically important indigenous species for WET testing, requiring extrapolation from reference species.

The ability to extrapolate toxicity test results to different species decreases with decreasing taxonomic relatedness (Suter *et al.* 1985, Suter and Rosen 1988). To reduce reliance on species extrapolation for WET testing, the sensitivity of seasonally available indigenous species can be compared to taxonomically related reference species. For most indigenous species that can be reared in the laboratory, WET tests would be limited to those addressing acute toxicity. Nonetheless, if a relationship between indigenous and reference species can be established, the site-specificity of regularly scheduled WET tests using reference species may be improved.

Species sensitivity comparisons are often based on establishing concentration-response relationships for a selected response. However, comparing the acute sensitivity of different animal species to a toxicant based on a single response assumes that the response indicates the same level of stress for each species and, 1) is the most sensitive, biologically relevant response or, 2) differences in concentration-response relationships are independent of response. Lethality is probably the most commonly tested acute response since it is readily identifiable for many species and its biological relevance is obvious. However, lethality is difficult to ascertain for many species, particularly for early life stages. Furthermore, the level

of toxicant exposure that kills an animal is greater than, and has an uncertain relationship to, the level of exposure that assures eventual toxicant related death.

Study objective

The objective of this study was to compare the acute toxicity of gold mill effluent to early life stages of several marine species. The source of the effluent was a proposed mine on the coast of southern Alaska. Effluent was modified to approximate the osmolality and pH of seawater. The sensitivities of two common reference species, mysid shrimp and sheepshead minnows, and related species that may encounter gold mill effluent in Alaskan waters were compared. Since the reference species do not occur in Alaskan waters, their suitability for WET testing of this effluent was of interest. To more thoroughly compare their sensitivities, concentration-response relationships were determined for immobility, paralysis, and lethality. In the process, some common assumptions of acute toxicity testing were examined, and an index was created to improve the resolution of concentration-response relationships.

Materials and Methods

Effluent sample

At the time of this study, permitting was underway for opening the Kensington gold mine, located 72 km north of Juneau, Alaska, USA. Effluent from this proposed mine was used in this study. Effluent was

produced by N.A. Degerstrom, Inc., Spokane, Washington, USA, using pilot scale froth flotation and cyanide leaching processes followed by cyanide neutralization using chlorine gas. The osmolality of the effluent was equivalent to that of 12 ppt seawater. Additional information on effluent production and effluent characterization are presented in Chapter 4.

An 18 L sample of effluent was stored in a collapsible, polyethylene container at 4°C, in the dark, with no air space. With each use of effluent, the container was further collapsed to eliminate air space. The time from production of the effluent until completion of the final toxicity test for this study was 8 mo (Table 1). This lengthy testing period limited interpretation of the study results to the conservative components of the effluent sample since the concentrations of some of the components and toxicity may have changed during storage.

Test animals

Recommended marine WET test species (U.S. Environmental Protection Agency 1993), or related species that are indigenous to the coast of southern Alaska were selected for study (Table 1). Indigenous species were collected as adults and reared. Tests were performed when a sufficient number of post-hatch animals were available. Gravid red king crab (*Paralithodes camtschaticus*) and northern shrimp (*Pandalus borealis*) were collected near Juneau and maintained in flow-through seawater in outdoor tanks until release of

Table 1. Species, age at testing, duration of effluent storage prior to testing, number of animals per replicate (rep.) and number of replicates per effluent concentration (concn.) for gold mill effluent toxicity tests. Effluent osmolality and pH were adjusted to equal that of 31 ppt seawater.

Species	Age (h, post hatch)	Effluent storage time (weeks)	Animals/ rep.	Rep./ concn.
Red king crab (*Paralithodes camtschaticus*)	< 16	17	10	4
Northern shrimp (*Pandalus borealis*)	3	19	6	3
Pacific herring (*Clupea harengus pallasi*)	< 24	22	8	4
Mysid shrimp (*Mysidopsis bahia*)	96	37	5	4
Sheepshead minnow (*Cyprinodon variegatus*)	72	38	5	4

larvae. Red king crab were collected by the Alaska Department of Fish and Game. Pacific herring (*Clupea harengus pallasi*) were collected near Ketchikan, Alaska by the National Marine Fisheries Service. Eggs from the Pacific herring were fertilized in the laboratory and incubated in a greenhouse in flow-through seawater using gentle aeration. After hatching, larvae of indigenous species were transferred directly from rearing water to test solutions of the same temperature and osmolality.

Reference species were obtained from commercial sources (Table 1). Juvenile mysid shrimp (*M. bahia*, Aquatic Biosystems, Ft. Collins, Colorado, USA) and larval sheepshead minnows (*C. variegatus*, Enviro Sciences, Carrollton, Texas, USA) were shipped overnight in seawater at the test salinity (31 ppt) and held under test conditions for 16 to 20 h before initiation of toxicity tests. Holding water was aerated and reference animals were fed *Artemia* sp. nauplii during the acclimation period.

Artemia sp. was also intended to monitor changes in effluent toxicity during the storage period. Toxicity tests with *Artemia* sp. were conducted upon receipt of the effluent sample and through the time of the first toxicity test for this study. However, effluent toxicity to *Artemia* sp. was low and results were highly variable. As such, use of *Artemia* sp. for monitoring effluent toxicity was discontinued.

Toxicity tests

With the exception of the modifications described below, acute toxicity of the effluent was assessed following standard protocols for WET tests (U.S. Environmental Protection Agency 1993). Modifications were made to conserve the limited volume of effluent, and to better simulate the natural conditions of the indigenous species. Toxicity tests were conducted for 24 h in 50 ml Teflon beakers containing 40 ml of solution (protocol states 200 ml in 250 ml chamber). Indigenous species were tested under natural lighting in a greenhouse (protocol states ambient laboratory lighting, 16:8 h light:dark). Test dates were April 6, April 23, and May 7 for red king crab, northern shrimp, and Pacific herring, respectively. For the indigenous species, beakers were placed in a constant temperature water bath set to the rearing water temperature which approximated the near-shore seawater temperature (5°C) at the time of testing (protocol states 20 or 25°C). Toxicity tests with reference species were conducted using a 16:8 h light:dark photoperiod under ambient laboratory lighting in a 20°C water bath. The number of animals per chamber and the number of replicate chambers per exposure concentration varied, depending on the number of available animals and the volume requirements of each species (Table 1, protocol states 10 animals/chamber, 2 replicates). Animals were not fed during the experiments and exposure solutions were not aerated. Solutions were not renewed for the indigenous species. In the experiments with reference species, it was necessary to renew the test solutions after 12 h to maintain adequate

concentrations of dissolved oxygen.

Toxicity tests were performed using osmolality and pH adjusted effluent (Table 2). Two to 4 d prior to each experiment, depending on the predictability of test organism availability, concentrated synthetic seawater stocks were added to an aliquot of the effluent sample. Synthetic seawater stocks were prepared with glass distilled water and reagent grade chemicals (Table 2). After addition of the stocks and up to initiation of each experiment, pH was gradually adjusted using 0.1 M HCl. The resulting solution equalled the osmolality and pH of 31 ppt seawater (pH = 8.0), and consisted of 94% effluent due to dilution with concentrated synthetic seawater.

An approximately linear dilution series of effluent was prepared using 31 ppt synthetic seawater, aged 1 to 2 wk (Table 2). Test solutions ranged from 4% to 94% effluent. Synthetic seawater was prepared from the same stocks that were used for adjusting the effluent osmolality and was used as an experimental control for all species. In addition, natural seawater controls were used for indigenous species since they were reared and hatched in natural seawater. Natural seawater was obtained from 24 m depth in Auke Bay, Alaska through the Juneau Center, School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, laboratory seawater system, and passed through sand and 5 μm filters. Synthetic seawater stocks for the red king crab experiment were prepared using a slightly different synthetic seawater formulation (Harrison *et al.* 1980) than that listed in Table 2. The formulation was changed after the red

Table 2. Preparation of synthetic seawater (modified from Bidwell and Spotte 1985) and effluent for gold mill effluent toxicity tests. Stock solutions were prepared separately and combined as listed to make synthetic seawater, or added to effluent to adjust the osmolality to equal that of 31 ppt seawater.

Solution	Ingredient	Amount
stock A	NaCl	247.2 g
	KCl	6.7 g
	$NaHCO_3$	1.8 g
	distilled H_2O	734.3 ml
stock B	$CaCl_2$ $2H_2O$	13.6 g
	$MgCl_2$ $6H_2O$	46.6 g
	distilled H_2O	36.7 ml
stock C	$MgSO_4$ $7H_2O$	62.9 g
	distilled H_2O	91.8 ml
osmolality and pH adjusted effluent	effluent	825 ml
	stock A	46.98 ml
	stock B	2.35 ml
	stock C	5.87 ml
	0.1 M HCl	(to pH 8.0)
synthetic seawater	distilled H_2O	800 ml
	stock A	79.25 ml
	stock B	3.96 ml
	stock C	9.91 ml
	0.1 M HCl	(to pH 8)

king crab experiment due to minor differences between red king crab behavior in synthetic seawater and natural seawater controls. These differences were not sufficient to violate test acceptability criteria (see below).

Effects of effluent on the behavior of the animals were recorded on 4 occasions between exposure durations of 2 and 12 h and after 24 h. Chambers were rotated to create a slight current and carry affected animals to the center so they could be observed, after settling, in a single field of view under a dissecting microscope at 6.7x magnification. Animals were not otherwise disturbed. Animals were observed for 2 to 3 min and classified as: 1) mobile, if they changed their location; 2) immobile, if they did not change their location but displayed movement; 3) paralyzed, if no movement was exhibited. The criterion for an acceptable experiment was mobility in $\geq$ 90% of the control animals.

After 24 h, test solutions were replaced by carefully pipetting out 90% of the solution and adding seawater. This procedure was repeated 3 times for each chamber. After seawater transfer, behavior was monitored until the behavior of animals in all chambers matched the behavior of control animals or until the condition of control animals began to decline. Animals that were paralyzed at the conclusion of the 24 h toxicity test and remained paralyzed during all observations after transfer to seawater were reclassified as dead for the 24 h observation. Red king crab were not transferred to seawater after the 24 h toxicity test since investigation of recovery was not

an original objective. Sheepshead minnows were not transferred to seawater because of a lack of response to the effluent.

Chemical analysis

The ionic composition of effluent was characterized and is presented in Chapter 4. Temperature, pH, and dissolved oxygen were measured after 1, 10 or 12, and 24 h in one replicate of each effluent exposure concentration using hand held meters (Table 3). Salinity, converted from osmolality, was measured with a vapor pressure osmometer within 1 h of the start and at the end of each experiment. Conversion was based on the salinity of seawater at the measured exposure solution osmolality.

Data analysis

The trimmed Spearman-Karber method was used to estimate 24 h median effect concentrations (24-h EC50s) for immobility and paralysis, and the median lethal concentration (24-h LC50) (Hamilton *et al.* 1977, U.S. Environmental Protection Agency 1994). Since an appropriate method was not found for determining the statistical significance of differences among three EC50 or LC50 values, conclusions were based on graphical interpretation.

In addition to graphical interpretations, concentration-response relationships were analyzed by comparing the response proportions (p) at each effluent concentration after 24 h. The arcsin transformation, $p' = \arcsin(p^{0.5})$, was used for statistical analyses to increase

Table 3. Temperature (Temp.), pH, dissolved oxygen (DO), and salinity of exposure solutions[a] for each gold mill effluent toxicity test. Species were tested at an early life stage. Effluent osmolality and pH were adjusted to equal that of 31 ppt seawater.

Species	Temp. (°C)	pH	DO (mg/L)	Salinity[b] (ppt)
Red king crab	5.9 (0.4, 20)	7.88 (0.12, 20)	7.3 (0.1, 13)	31.8 (0.3, 20)
Northern shrimp	5.1 (0.2, 21)	7.88 (0.11, 21)	7.3 (0.1, 7)	31.2 (0.2, 21)
Pacific herring	4.5 (0.3, 21)	7.78 (0.15, 21)	9.1 (0.1, 7)	31.9 (0.2, 21)
Mysid shrimp	20.1 (0.0, 28)	7.86 (0.06, 28)	5.4 (0.8, 28)	31.0 (0.3, 14)
Sheepshead minnow	19.7 (0.5, 28)	7.81 (0.06, 28)	5.4 (1.4, 28)	31.0 (0.1, 14)

[a]Values are arithmetic means (SD, n = pooled measurements for entire experiment). Where $n < 21$, equipment malfunction precluded planned measurement.
[b]Converted from osmolality. Equal to the salinity of seawater at the measured osmolality.

normality, homoscedasticity, and additivity of the response proportions (Zar 1996). Single classification, model I ANOVA was used to analyze differences in transformed response proportions among species at each effluent concentration. When differences were significant ($p \leq 0.05$), Tukey's tests were conducted for each pairwise comparison using SYSTAT 7.0 for Windows (SPSS Inc. 1997). Experimentwise error across concentrations was not controlled for. That is, the type I error rate of all comparisons taken together was greater than $\alpha = 0.05$.

For comparing the toxicity of each exposure concentration over time, a response index was created, incorporating immobility and paralysis proportions into a single quantity. Death was not included since the definition of death was dependent on recovery after 24 h of effluent exposure, and recovery at intermediate durations was not assessed. The response index (RI) was continuous between 0 and 1 and defined as:

$$RI = (A/2 + B)/n$$

where A is the number of immobile (not including paralyzed) animals in a chamber, B is the number of paralyzed animals, and n is the total number of animals in the chamber.

The response index was created to allow presentation of the immobility and paralysis data for each species in a single figure. The response index differentiated exposures that resulted in the same

proportion of one response, but a different proportion of the other response. The response index was useful for comparing trends over time and ranking the severity of response across concentrations for a species. It was not an absolute measure of response severity and was not intended for comparing species. As presented above, it was equivalent to the average of the proportions of immobile and paralyzed animals. The denominator of 2 arbitrarily assigned the severity of paralysis as twice that of immobility.

Results

The rank and magnitude of differences in species sensitivity after 24 h exposure to gold mill effluent depended on the response (Figure 1, Table 4, Appendices 10 and 11). The sensitivity of reference species bracketed that of indigenous species for all responses, with mysid shrimp and sheepshead minnows being the most and least sensitive species, respectively. However, based on immobility and paralysis, the three crustacean species were more sensitive than the two fish species and based on lethality, Pacific herring were the second most sensitive, after mysid shrimp. Effluent was not lethal to northern shrimp or sheepshead minnows. Lethality is not known for red king crab since recovery was not investigated.

For crustaceans, there was a clear gradation of responses across effluent concentrations (Figure 2, Table 4). Effects on red king crab and northern shrimp were generally stable by 3 to 6 h. The responses of mysid shrimp stabilized or increased over time, depending on the

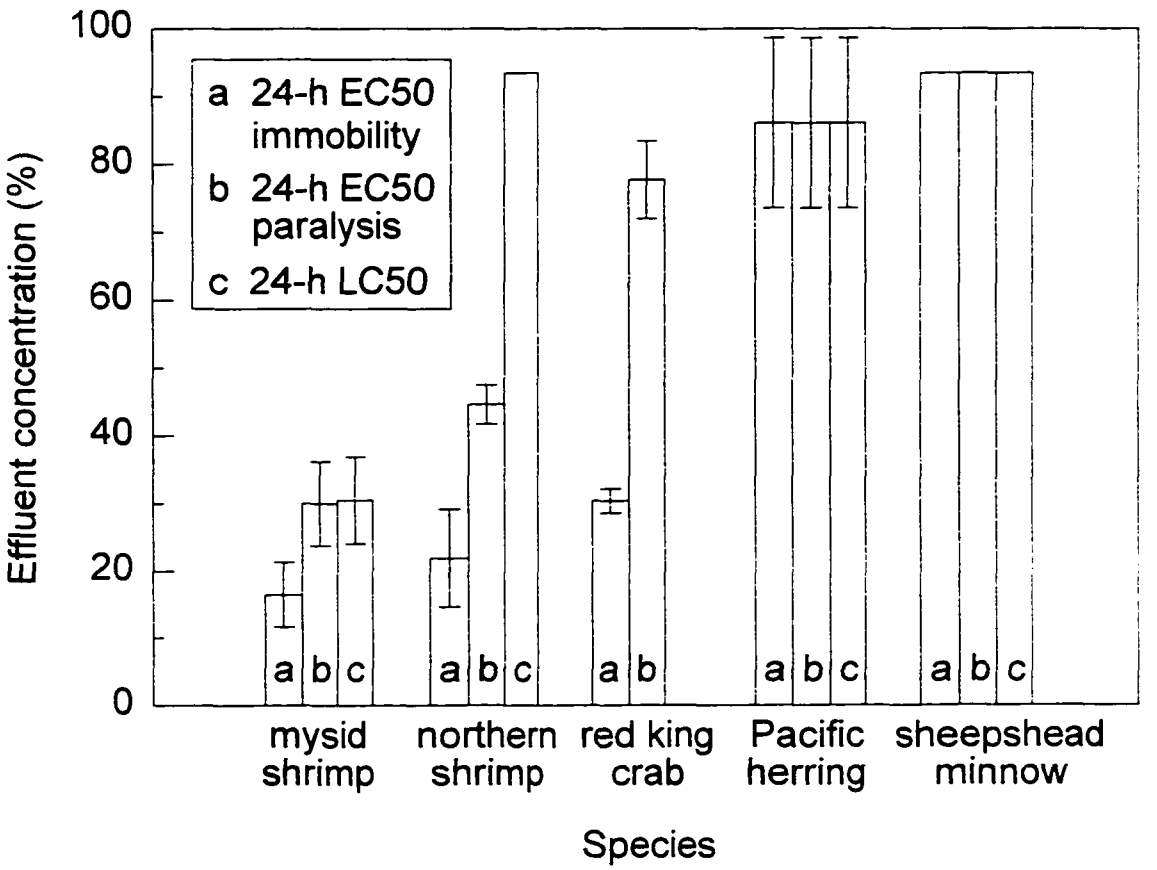

Figure 1. 24-h median effect concentrations (24-h EC50) and median lethal concentrations (24-h LC50) for early life stage fish and crustaceans exposed to gold mill effluent. The 24-h LC50 for red king crab was not determined. Effluent osmolality and pH were adjusted to equal that of 31 ppt seawater. Error bars are 95% confidence intervals (trimmed Spearman-Karber method). No error bars indicate < 50% response at the highest (94%) effluent concentration (non-existent median response concentration). Refer to Appendix 10 for data.

Table 4. Mean proportions (Prop.) of early life stage animals that responded after 24 h at each gold mill effluent concentration (Eff.). Effluent osmolality and pH were adjusted to equal that of 31 ppt seawater. Proportions were ranked from highest to lowest for each response within each concentration. Species (Spec.): ms, mysid shrimp; rkc, red king crab; ns, northern shrimp; sm, sheepshead minnow; Ph, Pacific herring. Mean proportions for species that do not share a superscripted character were significantly different (Tukey's, $p \leq 0.05$). Experimentwise error rate across concentrations was not controlled for. There were no significant differences at < 37% effluent with the exception of ms > rkc for the immobility proportion at 21% effluent. Death was not determined for red king crab.

	Immobility			Paralysis			Death	
Eff. (%)	Spec.	Prop.	(SD)	Spec.	Prop.	(SD)	Spec.	Prop. (SD)
37	ms^{a}	1.00	(0.00)	ms^{a}	0.60	(0.03)	ms^{a}	0.60 (0.03)
	rkc^{ab}	0.96	(0.05)	Ph^{b}	0.04	(0.05)	Ph^{b}	0.04 (0.05)
	ns^{bc}	0.62	(0.04)	ns^{b}	0.02	(0.06)	ns^{b}	0.00 (0.00)
	sm^{cd}	0.29	(0.04)	rkc^{b}	0.00	(0.00)	sm^{b}	0.00 (0.00)
	Ph^{d}	0.04	(0.05)	sm^{b}	0.00	(0.00)		
54	ms^{a}	1.00	(0.00)	ms^{a}	1.00	(0.00)	ms^{a}	0.99 (0.05)
	ns^{a}	1.00	(0.00)	ns^{a}	0.98	(0.06)	Ph^{b}	0.17 (0.08)
	rkc^{a}	0.93	(0.13)	rkc^{b}	0.19	(0.09)	ns^{c}	0.00 (0.00)
	Ph^{b}	0.17	(0.08)	Ph^{b}	0.17	(0.08)	sm^{c}	0.00 (0.00)
	sm^{b}	0.15	(0.08)	sm^{b}	0.00	(0.00)		
71	ms^{a}	1.00	(0.00)	ms^{a}	1.00	(0.00)	ms^{a}	1.00 (0.00)
	ns^{a}	1.00	(0.00)	ns^{a}	1.00	(0.00)	Ph^{b}	0.31 (0.01)
	rkc^{a}	1.00	(0.00)	Ph^{b}	0.31	(0.01)	ns^{c}	0.00 (0.00)
	Ph^{b}	0.31	(0.01)	rkc^{b}	0.15	(0.08)	sm^{c}	0.00 (0.00)
	sm^{b}	0.15	(0.08)	sm^{c}	0.00	(0.00)		

continued.

Table 4 continued.

	Immobility		Paralysis		Death	
Eff. (%)	Spec.	Prop. (SD)	Spec.	Prop. (SD)	Spec.	Prop. (SD)
94	ms[a]	1.00 (0.00)	ms[a]	1.00 (0.00)	ms[a]	1.00 (0.00)
	ns[a]	1.00 (0.00)	ns[a]	1.00 (0.00)	Ph[b]	0.60 (0.08)
	rkc[a]	1.00 (0.00)	rkc[ab]	0.89 (0.06)	ns[c]	0.00 (0.00)
	Ph[b]	0.60 (0.08)	Ph[b]	0.60 (0.08)	sm[c]	0.00 (0.00)
	sm[b]	0.45 (0.01)	sm[c]	0.00 (0.00)		

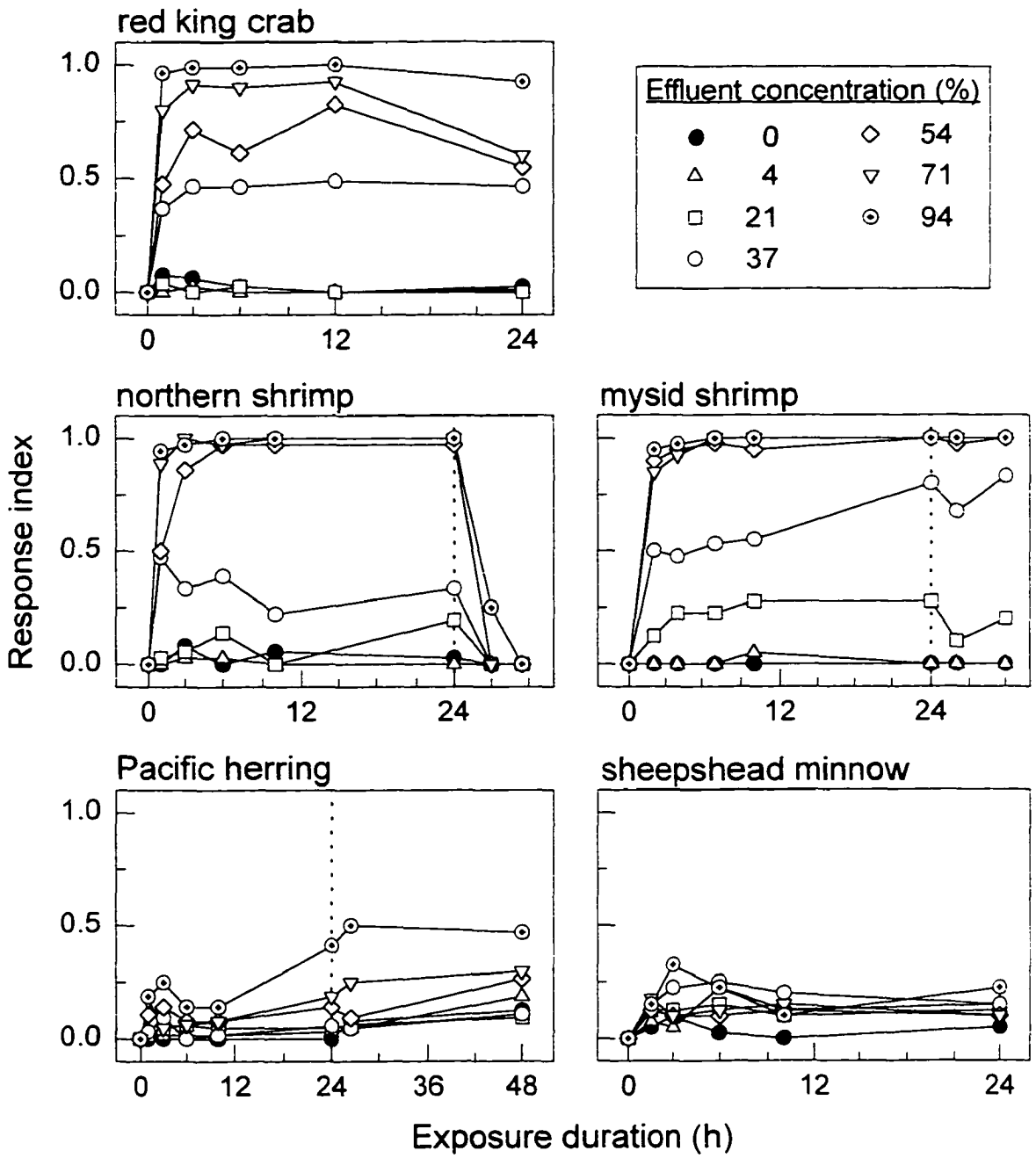

Figure 2. Toxicity across time at each exposure concentration for early life stage fish and crustaceans exposed to gold mill effluent. Effluent osmolality and pH were adjusted to equal that of 31 ppt seawater. The response index incorporated the proportions of immobile and paralyzed animals (0 = all animals mobile, not toxic, 1 = all animals paralyzed, most toxic). Error bars are ± 1 SE. Vertical dashed lines indicate the time of transfer from effluent to seawater. Red king crab and sheepshead minnows were not transferred to seawater.

effluent concentration. Most paralyzed mysid shrimp fragmented during the seawater transfer, confirming death. Recovery was evident in some immobilized mysid shrimp, but cannibalization by mobile mysid shrimp prevented continued assessment of recovery.

Effects on fish were more variable than for crustaceans and increased through 24 h for Pacific herring (Figure 2, Table 4). No movement was observed in immobile Pacific herring and none recovered after transfer to seawater. As such, all three 24 h median response concentrations were equal (Figure 1). Monitoring of Pacific herring recovery was discontinued 24 h after transfer to seawater since control animals were beginning to display symptoms of stress. Sheepshead minnows were, overall, the most tolerant species included in this study. There were some immobile sheepshead minnows in 94% effluent, particularly after 3 h exposure (Figure 2). However, the concentration-response relationship was highly variable. Responses were insufficient to calculate 24-h median response concentrations (Figure 1). Recovery was not investigated due to a lack of response and high variability in the controls.

Discussion

Species comparisons

Taxonomically, mysid shrimp are somewhat less related to the indigenous crustaceans than are sheepshead minnows to Pacific herring (Figure 3). Similarity in sensitivity to effluent generally increased with taxonomic similarity (Table 4, Figures 1 and 2) in accord with

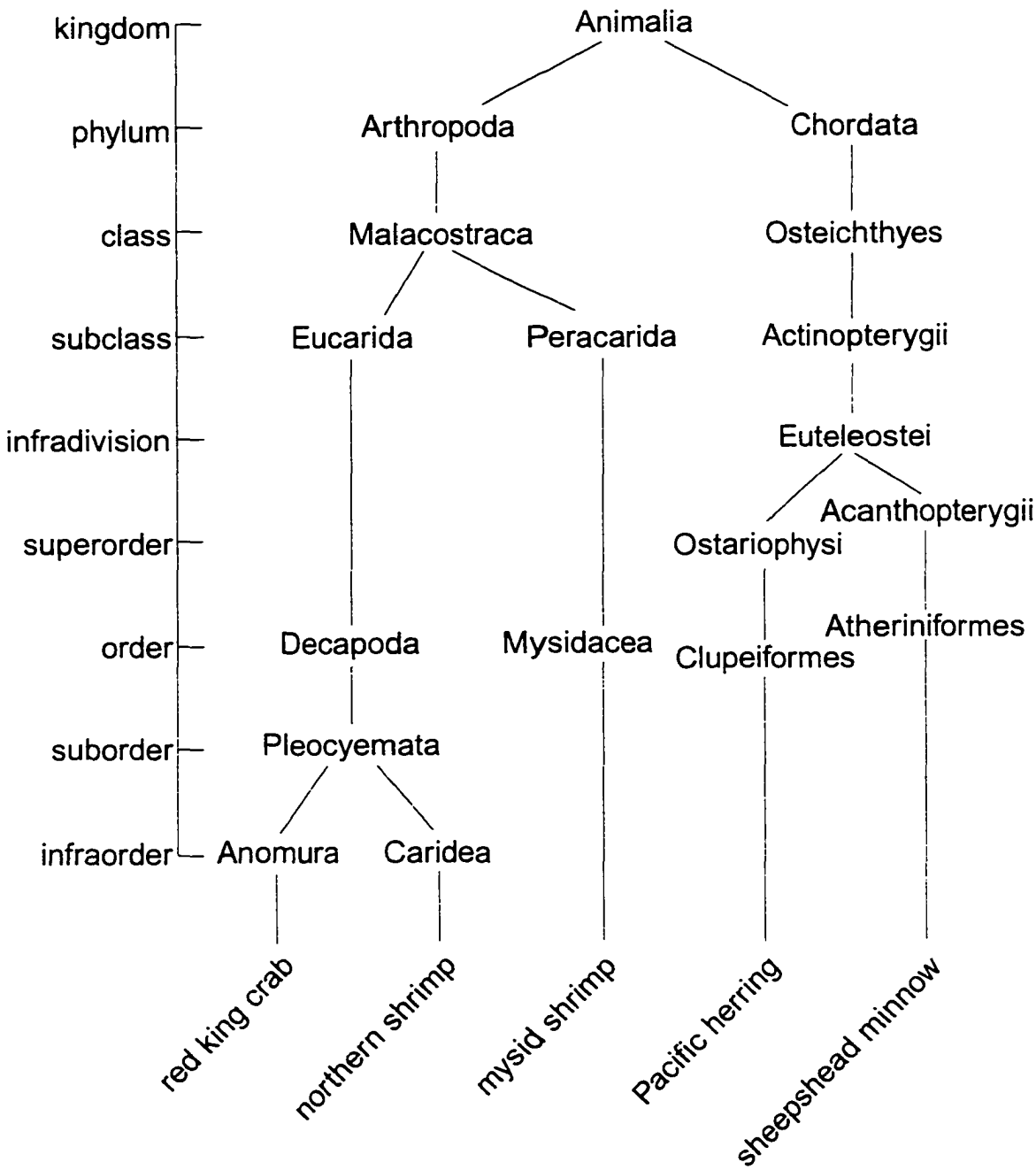

Figure 3. Taxonomic relationships of early life stage fish and crustaceans used in gold mill effluent toxicity tests (classifications from Perlmutter 1961, Nelson 1984, Schram 1986, Kozloff 1987, Lassuy 1989).

findings for other species and toxicants (Suter *et al.* 1985, Suter and Rosen 1988). As a whole, crustaceans displayed greater sublethal sensitivity and clearer distinctions between the responses compared to fish. Since only two fish species were included and sheepshead minnows were not visibly affected, generalizations regarding fish are poorly supported. However, responses of different marine fish species to toxicants have been found to be more similar than responses of different marine crustacean species (Suter and Rosen 1988). The greatest contradiction to the general finding of like species exhibiting like responses was the occurrence of lethality in mysid shrimp and Pacific herring but not in the other species.

Effluent toxicity to a single species was not monitored over the time period that these experiments were conducted (Table 1). As such, the possibility existed that some of the differences in the sensitivity of the species could have been related to the time since effluent production (Table 1). *Artemia* sp. were intended to be used for monitoring effluent toxicity. Use of *Artemia* sp. for this purpose was discontinued due to high variability in response and a lack of sensitivity to the effluent.

The rapid response of northern shrimp and red king crab to effluent and rapid recovery of northern shrimp after transfer to seawater (Figure 2) suggested the possibility of avoidance of unpreferred but tolerable conditions. In support of this suggestion, red king crab larvae have been found to cease swimming to adjust their position in a salinity gradient and resume swimming after encountering

water of preferable salinity (Shirley and Shirley 1989). The response of the indigenous crustaceans could also have been interpreted as narcosis; however, narcosis is generally attributed to organic compounds (Veith *et al.* 1983, Schultz 1989).

The sensitivity of the two reference species bracketed the sensitivity of the indigenous species for all responses (Figure 1), indicating that mysid shrimp (the most sensitive) may serve as a conservative surrogate species for monitoring toxicity of this effluent while sheepshead minnows (the least sensitive) may not. However, effluent toxicity to mysid shrimp resulted from the major seawater ion content (Chapter 4), an effluent component that poses little risk of toxicity in marine receiving waters. Recall that the osmolality of the effluent was equivalent to 12 ppt seawater. Since sheepshead minnows are more tolerant to toxicity resulting from unique ratios of major seawater ions than *M. bahia* (Price *et al.* 1990), they may be better suited for monitoring toxicity of effluents with elevated concentrations of total dissolved solids. Sheepshead minnows are, however, generally less sensitive to chemical exposure than *M. bahia* (Suter and Rosen 1988).

Exposure duration is another factor that can influence species sensitivity comparisons. A 24 h exposure duration was selected for this study to conserve the limited volume of effluent and because actual exposure durations in marine waters would be brief (U.S. Department of Agriculture 1991). The minimum recommended duration for acute WET tests is 24 h (U.S. Environmental Protection Agency 1993),

while 48 or 96 h exposures are probably more typical. 24 h was sufficient for the indigenous crustaceans since their responses became asymptotic. However, responses of Pacific herring and mysid shrimp did not become asymptotic (Figure 2). As such, concluding the experiments at 24 h may have resulted in an incomplete comparison of acute sensitivity.

Implications of effluent storage and adjustment

During an actual milling operation, effluent may be treated prior to discharge. For the effluent used in this study, only cyanide destruction was performed. Effluent treatment commonly includes retention in a holding pond to allow volatilization and degradation of nonconservative effluent components, and precipitation and removal of suspended solids and metals (Read and Manser 1976, Simovic and Snodgrass 1985, Scott 1989). Due to the considerable length of effluent storage, this study addressed toxicity of conservative effluent components. It is possible that the effect of storage on effluent composition and toxicity may have been similar to the effect of retention in a holding pond. However, this relationship could not be determined since a mill that produced this effluent was not in operation.

Addition of concentrated synthetic seawater and adjustment of pH undoubtedly decreased toxicity that would have resulted from osmotic and pH stress, particularly for the indigenous species since they were stenohaline relative to the reference species. Justification for

adjustment of osmolality was to test for toxicity of effluent components rather than for an osmotic response. For a marine discharge, osmotic responses are not a regulatory issue since they are unlikely to be the cause of toxicity in receiving waters. Justification for pH adjustment was that direct effects of pH on toxicity were considered unlikely due to the high buffering capacity of seawater. However, modification of osmolality and pH may have affected the toxicity of other effluent components (Chapman *et al.* 1982).

Risk of marine toxicity

Regardless of the effects of storage and adjustment of osmolality and pH, it appears unlikely that the life stages of the species included in this study would be affected by discharge of this effluent. This statement is based on exposure risk as much as it is based on the results of the toxicity studies. Effluent would have to be toxic at much lower concentrations than were found in this study, or be discharged at a high rate relative to the residence time of receiving waters to cause acute toxicity.

Fresh or brackish effluent that is discharged directly to the ocean may be released near-shore, below the surface, through a diffuser, and rise as a buoyant plume. Depending on the depth of discharge, the density differential between effluent and seawater, and water column stratification, effluent will settle as a layer below or at the surface (Proni *et al.* 1994). After settling, dispersion

depends on large scale water movement and turbulence (Koh and Brooks 1975). Maximum measured effluent concentrations in plumes from three large municipal outfalls ranged from < 1% near the diffuser of one outfall to 7% in a surface boil of another outfall (Washburn *et al.* 1992, Proni *et al.* 1994). For a marine discharge, the effluent used in this study was predicted to have a maximum settled concentration of < 1% effluent in a 7.2 m diameter layer. This layer was predicted to form 24 m above the diffuser and 76 m below the sea surface (U.S. Department of Agriculture 1991).

The effects of mine drainage, undiverted surface water, and treated sewage that are likely to comprise a portion of whole effluent from a mining operation were not investigated in this study. Any contribution to whole effluent toxicity from mine drainage would probably depend on the acid generating capacity of the rock (see Kelley and Tuovinen 1988) and the resulting dissolved metal concentrations. Undiverted surface water would probably decrease whole effluent toxicity due to dilution. Treated sewage would probably have little effect on toxicity since it is likely to comprise only a minor portion of whole effluent from a mining operation (e.g. U.S. Department of Agriculture 1991). This study investigated the toxicity of the mill portion of whole effluent, which is potentially one of the more toxic components.

Response-dependent differential sensitivity

Including more than one response in species sensitivity comparisons may lead to different conclusions than would have been drawn had a single response been investigated. For this study, an overall ranking of sensitivity of the indigenous species could not be made since ranking depended on the response being measured (Figure 1). Including three responses increased confidence in the conclusion that mysid shrimp were the most sensitive and sheepshead minnows were the least sensitive species since their rankings were consistent for each response. However, the magnitude of differences still depended on the response that was measured.

Similar findings have been demonstrated in other studies addressing multiple responses to toxicants. The magnitude of the difference in the sensitivity of three marine amphipod species to tributyltin was twice for mortality what it was for reburial behavior (Meador *et al.* 1993). Response-dependence was attributed to differential rates of toxicant uptake, resulting in an absence of the reburial response in species that more rapidly accumulated and succumbed to the toxicant. As another example, the rank of sensitivity was reversed for two species of marine fish exposed to an organophosphate insecticide depending on the response: mortality or acetylcholinesterase inhibition (Van Dolah *et al.* 1997).

In the present study, the physiological implications of immobility and paralysis differed among the species, further complicating the species comparisons (Figure 2). The sublethal

responses coincided with death in Pacific herring, and paralysis assured death in mysid shrimp, while immobility and paralysis were reversible in northern shrimp. The relationship between some sublethal stress responses and other organism level responses has been determined for some species (Moore and Dillon 1993, Conroy *et al.* 1996) and linked to population effects for others (Nisbet *et al.* 1989, Sibley *et al.* 1997). As in the current study, these relationships were species specific.

Two studies of metal toxicity to terrestrial nematodes serve to illustrate the importance of response-dependence and the biological implications of stress responses when comparing species sensitivity. In these studies, the reproductive period was the most sensitive response investigated. When sensitivity of the two species to copper was compared, both were equally susceptible to changes in population growth rate, yet their EC20 values for reproductive impairment differed (Kammenga and Risken 1996). When one of these species was exposed to cadmium, a considerable reduction in the reproductive period had no effect on population growth. However, an effect on length of the juvenile period, the least sensitive response, resulted in decreased population growth (Kammenga *et al.* 1996).

The response index was a simple attempt to integrate responses (Figure 2). Since the actual magnitude of the difference in physiological stress indicated by immobility or paralysis for each species was not known, the index could not be interpreted as an absolute measure of stress and could not be used to compare species.

Conceptually, a stress index could be created, representing a linear scale of increasing stress, with a value of 0 equalling the stress level of control animals and a value of 1 representing death. The index value of intermediate stress responses would be based on their relation to population level effects.

Such a stress index would be a first step toward understanding the biological relevance of stress responses. Another important step would be to quantify the ecological implications of behavioral responses. Immobility or paralysis are clearly adverse for any species due to an inability to feed, avoid predation, and maintain an optimal position in the water column. However, similar behavior between species may not indicate similar ecological risk.

Conclusions

For sublethal responses, gold mill effluent was more toxic to the 3 crustacean species included in this study than the 2 fish species; however, the effluent was lethal only to mysid shrimp and Pacific herring. For all responses, sensitivity of the 2 reference species bracketed that of the 3 indigenous species, with mysid shrimp being the most sensitive and sheepshead minnows being the least sensitive. The magnitude of differences and the rank of sensitivity for the indigenous species depended on the response that was measured. As such, an overall ranking of species sensitivity could not be made, demonstrating that a single response, including lethality, can be insufficient when comparing the acute sensitivity of species to a

toxicant. When transferred to seawater, northern shrimp regained mobility after being paralyzed by effluent. Mysid shrimp and Pacific herring did not regain mobility, illustrating the need to understand the biological relevance of sublethal responses. For the life stages and species included in this study, it is unlikely that exposure to this gold mill effluent in the ocean could be sufficient to be cause acute toxicity.

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Literature Cited

Bidwell, J.P. and S. Spotte. 1985. *Artificial Seawaters: Formulas and Methods*. Jones and Bartlett Publ., Boston, MA, USA, 349 pp.

Chapman, P.M., M.A. Farrell, and R.O. Brinkhurst. 1982. Relative tolerances of selected aquatic oligochaetes to combinations of pollutants and environmental factors. *Aquat. Toxicol.* 2:69-78.

Coldwell, J.R. and E.C. Gensler. 1993. Potential for submarine tailings disposal to affect the availability of minerals from United States coastal areas. U.S. Bureau of Mines, OFR 101-93, 49 pp.

Conroy, P.T., J.W. Hunt, and B.S. Anderson. 1996. Validation of a short-term toxicity test endpoint by comparison with longer-term effects on larval red abalone *Haliotis rufescens*. *Environ. Toxicol. Chem.* 15:1245-1250.

Ellis, D.V., T.F. Pedersen, G.W. Poling, C. Pelletier, and I. Horne. 1995. Review of 23 years of STD: Island Copper Mine, Canada. *Mar. Geores. Geotech.* 13:59-99.

Hamilton, M.A., R.C. Russo, and R.V. Thurston. 1977. Trimmed Spearman-Karber method for estimating lethal concentrations in toxicity bioassays. *Environ. Sci. Technol.* 11:714-719.

Harrison, P.J., R.E. Waters, and F.J.R. Taylor. 1980. A broad spectrum artificial seawater medium for coastal and open ocean phytoplankton. *J. Phycol.* 16:28-35.

Kammenga, J.E. and J.A.G. Riksen. 1996. Comparing differences in species sensitivity to toxicants: phenotypic plasticity versus concentration-response relationships. *Environ. Toxicol. Chem.* 15:1649-1653.

Kammenga, J.E., M. Busschers, N.M. Van Straalen, P.C. Jepson, and J. Bakker. 1996. Stress induced fitness reduction is not determined by the most sensitive life-cycle trait. *Funct. Ecol.* 10:106-111.

Kelley, B.C. and O.H. Tuovinen. 1988. Microbial oxidations of minerals in mine tailings. *Chemistry and Biology of Solid Waste: Dredged Material and Mine Tailings*. W. Salomons and U. Forstner, eds., Springer-Verlag, Berlin, Germany, pp. 33-53.

Koh, R.C.Y. and N.H. Brooks. 1975. Fluid mechanics of waste-water disposal in the ocean. *Ann. Rev. Fluid Mech.* 7:187-211.

Kozloff, E.N. 1987. *Marine Invertebrates of the Pacific Northwest*. University of Washington Press, Seattle, WA, USA. 511 pp.

Lassuy, D.R. 1989. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (Pacific Northwest), Pacific herring. *U.S. Fish and Wildlife Service, Biological Report* 82(11.126), 18 pp.

McKim, J.M. 1977. Evaluation of tests with early life stages of fish for predicting long-term toxicity. *J. Fish. Res. Board Can.* 34:1148-1154.

Meador, J.P., U. Varanasi, and C.A. Krone. 1993. Differential sensitivity of marine infaunal amphipods to tributyltin. *Mar. Biol.* 116:231-239.

Moore, D.W. and T.M. Dillon. 1993. The relationship between growth and reproduction in the marine polychaete *Nereis (Neanthes) arenaceodentata* (Moore): implications for chronic sublethal sediment bioassays. *J. Exp. Mar. Biol. Ecol.* 173:231-246.

Nelson, J.S. 1984. Fishes of the World. 2nd ed., John Wiley and Sons, New York, NY, USA.

Nisbet, R.M., W.S.C. Gurney, W.W. Murdoch, and E. McCauley. 1989. Structured population models: a tool for linking effects at individual and population level. *Biol. J. Linnean Soc.* 37:79-99.

Perlmutter, A. 1961. *Guide to Marine Fishes*. New York University Press, NY, USA. 431 pp.

Poling, G.W. and D.V. Ellis. 1995. Importance of geochemistry: the Black Angel lead-zinc mine, Greenland. *Mar. Geores. Geotech.* 13:101-118.

Price, E.E., M.J. Donahue, K.L. Dickson, and J.H. Rodgers, Jr. 1990. Effects of elevated calcium concentration on Na-K-ATPase activity of two euryhaline species, *Cyprinodon variegatus* and *Mysidopsis bahia*. *Bull. Environ. Contam. Toxicol.* 44:121-128.

Proni, J.R., H. Huang, and W.P. Dammann. 1994. Initial dilution of southeast Florida ocean outfalls. *J. Hydr. Engrg.* 120:1409-1425.

Read, A.D. and R.M. Manser. 1976. Mineral floatation - a study of the re-use and disposal of aqueous solutions containing organic reagents. *Water Res.* 10:243-251.

Schram, F.R. 1986. *Crustacea*. Oxford University Press, New York, NY, USA. 606 pp.

Schultz, T.W. 1989. Nonpolar narcosis: a review of the mechanism of action for baseline aquatic toxicity. *Aquatic Toxicology and Hazard Assessment*. U.M. Cowgill and L.R. Williams, eds., American Society for Testing and Materials, Philadelphia, PA, USA, ASTM STP 1027, 12th Volume, pp. 104-109.

Scott, J.S. 1989. An overview of goldmill effluent treatment. *Proceedings Gold Mining Effluent Treatment Seminars*, Vancouver BC, Canada, Environment Canada, pp. 1-22.

Shirley, T.C. and S.M. Shirley. 1989. Temperature and salinity tolerances and preferences of red king crab larvae. *Mar. Behav. Physiol.* 16:19-30.

Sibley, P.K., D.A. Benoit, and G.T. Ankley. 1997. The significance of growth in *Chironomus tentans* sediment toxicity tests: relationship to reproduction and demographic endpoints. *Environ. Toxicol. Chem.* 16:336-345.

Simovic, L. and W.J. Snodgrass. 1985. Natural removal of cyanides in gold milling effluents - evaluation of removal kinetics. *Water Poll. Res. J. Canada* 20:120-135.

SPSS, Inc. 1997. SYSTAT 7.0. Chicago, IL, USA

Suter, G.W., II., L.W. Barnthouse, J.E. Breck, R.H. Gardner, and R.V. O'Neill. 1985. Extrapolating from the laboratory to the field: how uncertain are you? *Aquatic Toxicology and Hazard Assessment: 7th Symposium*. R.D. Cardwell, R. Purdy and R.C. Bahner, eds., American Society for Testing and Materials, Philadelphia, PA, USA, ASTM STP 854, pp. 400-413.

Suter, G.W.I. and A.E. Rosen. 1988. Comparative toxicology for risk assessment of marine fishes and crustaceans. *Environ. Sci. Tech.* 22:548-556.

U.S. Department of Agriculture. 1991. *Draft Environmental Impact Statement Kensington Gold Project*. Juneau Ranger District, Juneau, AK, USA.

U.S. Environmental Protection Agency. 1993. *Methods for measuring the acute toxicity of effluents to freshwater and marine organisms*. 4th ed., EPA/600/4-90/027F.

U.S. Environmental Protection Agency. 1994. US EPA toxicity data analysis software. James Lazorchak, US EPA, EMSL, EMRD, 3411 Church St., Cincinnati, OH 45244.

Van Dolah, R.R., P.C. Macer, M.A. Fulton, and G.I. Scott. 1997. Comparison of azinphosmethyl toxicity to juvenile red drum (*Sciaenops ocellatus*) and the mummichog (*Fundulus heteroclitus*). *Environ. Toxicol. Chem.* 16:1488-1493.

Veith, G.D., D.J. Call, and L.T. Brooke. 1983. Structure-toxicity relationships for the fathead minnow, *Pimephales promelas*: narcotic industrial chemicals. *Can. J. Fish. Aquat. Sci.* 40:743-748.

Washburn, L., B.H. Jones, A. Bratkovich, T.D. Dickey, and M.S. Chen. 1992. Mixing, dispersion, and resuspension in vicinity of ocean wastewater plume. *J. Hydr. Engrg.* 118:38-59.

Woltering, D.M. 1984. The growth response in fish chronic and early life stage toxicity tests: a critical review. *Aquat. Toxicol.* 5:1-21.

Zar, J.H. 1996. *Biostatistical Analysis* 3rd ed., Prentice-Hall, Upper Saddle River, NJ, USA, 662 pp.

Chapter 4

The Role of Calcium and Sodium in Toxicity of Gold Mill Effluent to Mysid Shrimp (*Mysidopsis bahia*)

Abstract

The source of acute toxicity of a sample of gold mill effluent to mysid shrimp (*Mysidopsis bahia*) was identified. Effluent osmolality was equivalent to that of 12 ppt seawater. At 5 effluent concentrations ranging from 4% to 100%, using 12 ppt seawater for dilution, maximum toxicity occurred at 37% effluent. Simulated effluent was created by adding Na^+, Ca^{2+}, Mg^{2+}, K^+, Cl^-, and SO_4^{2-} to distilled water at concentrations equal to those measured in the effluent. The unusual finding of greater toxicity at 37% than at 100% effluent was duplicated with simulated effluent, indicating that the source of effluent toxicity was 1 or more of the 6 ions that were included in the simulated effluent. Excess Ca^{2+} and Na^+ deficiency were among the greatest differences in the ionic composition of effluent relative to seawater. Toxicity decreased when the concentration of Ca^{2+} was decreased in simulated effluent. Toxicity increased when Na^+ was added to effluent. When the data from all experiments were combined and ion concentrations were presented as proportions relative to seawater, Ca^{2+} and the interaction of Ca^{2+} and Na^+ explained 76% of the variation in toxicity using multiple regression. Ca^{2+} was the source of effluent toxicity, and Na^+ deficiency reduced Ca^{2+} toxicity.

Introduction

Extraction of metals from ore may require using large volumes of acids, bases, and buffers. These reagents may contribute to high concentrations of total dissolved solids (TDS) in ore processing effluent, relative to freshwater. The ratio of ions that constitute TDS in ore processing effluent are likely to differ from the ratio in seawater. Other waters with high TDS and unique ion ratios include produced water from oil and gas production (Ho and Caudle 1997), irrigation drain water (Ingersoll *et al.* 1992), oil shale leachates (Meyer *et al.* 1985), seafood canning factories (Mendez *et al.* 1995), and desalination plants (Altayran and Madany 1992). For marine discharge of effluent, TDS is unlikely to cause toxicity in receiving waters or to be regulated.

Whole effluent toxicity (WET) tests (e.g. U.S. Environmental Protection Agency 1993a) and toxicity identification evaluation (TIE) methods (U.S. Environmental Protection Agency 1991) may be used to monitor and identify sources of effluent toxicity. TDS may contribute to toxicity in WET tests and may interfere with TIE studies. The mysid shrimp (*Mysidopsis bahia*) is an estuarine crustacean that is commonly used for marine WET tests and TIE studies. Although mysid shrimp are euryhaline (de Lisle and Roberts 1987), they have been found to be sensitive to ionic ratios that differ from that of seawater (Douglas and Horne 1997, Sauer *et al.* 1997).

Several methods have been used to account for TDS effects in studies of effluent toxicity to estuarine and marine organisms

(Burgess *et al.* 1995, Florida Dept. of Environmental Protection 1995, Ho *et al.* 1995, Douglas and Horne 1997, Sauer *et al.* 1997). The most appropriate method depends on the TDS of effluent, the ratios of ions in effluent relative to seawater, the salinity tolerance range of the test species, and the study objectives. To understand the effects of TDS and unique ion ratios on effluent toxicity, the individual and combined roles of ions must be understood.

Study objective

The objective of this study was to isolate the roles in toxicity to juvenile mysid shrimp (*M. bahia*) of ions that constituted the majority of TDS of a gold mill effluent. Mysid shrimp were the most sensitive to the same gold mill effluent in a related study that included larvae of several marine species (Chapter 3). In the related study (Chapter 3), the osmolality of the effluent was increased to equal that of seawater. Since the osmolality of the unadjusted effluent was equivalent to that of 12 ppt seawater, and the effect of osmolality adjustment on toxicity of the effluent was unknown, the current study was undertaken.

Materials and Methods

Effluent sample

At the time of this study, permitting was underway for opening the Kensington gold mine, located 72 km north of Juneau, Alaska, USA. Effluent from this proposed mine was used in this study. Effluent was

produced by N.A. Degerstrom, Inc., Spokane, Washington, USA using pilot scale froth flotation and concentrate leaching processes. Potassium amyl xanthate (PAX) and methyl isobutyl carbinol were used for the flotation process. Sodium cyanide and lead nitrate were used to leach the mineral concentrate. The majority of cyanide in the leaching process wastewater was neutralized by sparging with chlorine gas before being combined with the flotation process wastewater. During the leaching and cyanide neutralization steps, calcium oxide was used to maintain suitable pH for the process reactions. The flotation wastewater and cyanide leach wastewater were mixed in proportions representative of mill effluent from full scale operation.

An 18 L sample of effluent was stored in a collapsible, polyethylene container at 4°C, in the dark, with no air space. With each use of effluent, the container was further collapsed to eliminate air space. Due to an initial focus on other species, effluent toxicity tests using mysid shrimp were conducted between 8.5 and 15 mo after effluent production. Because of this lengthy storage period, conclusions pertaining to effluent toxicity were restricted to the major ion content of the effluent. Major ions refer to those that comprise greater than 1% of the total molar weight of the ionic components of seawater.

It was suspected that significant concentrations of other effluent components, including PAX and cyanide, were lost from solution during storage. Major ion concentrations were assumed to be stable because the osmolality and pH of the effluent were stable

throughout the storage period. Regardless of the validity of this assumption, the results of this study can be interpreted with respect to the major ion content that was measured at the time of the experiments.

Specifications pertaining to all experiments

Mysid shrimp were shipped overnight from a commercial source (Aquatic Biosystems, Ft. Collins, Colorado, USA) at 2 d post hatch and used for toxicity tests at age 4 d as juveniles. The commercial source conducted monthly reference toxicity tests using KCl to assure uniformity in organism health. Mysid shrimp were shipped in seawater at the test salinity, or acclimated over a 5 h period to a range of salinities for some of the experiments. The maximum change in salinity during the 5 h acclimation period was from 20 ppt to 8 ppt. After acclimation, mysid shrimp were held in seawater at test salinities and conditions for 16 to 20 h before initiation of experiments. Holding water was aerated and mysid shrimp were fed *Artemia* sp. nauplii during the acclimation and holding periods. Mysid shrimp were not fed during experiments.

The preparation of all solutions and rationale for the experiments are summarized in Table 1. All exposure solutions were adjusted to pH 8.0. Experiment durations ranged from 3 to 24 h. Experiments were conducted under ambient laboratory lighting in a 20°C water bath in 50 ml Teflon beakers containing 40 ml of solution. A 16:8 h light:dark photoperiod was used for 24 h experiments. Four

Table 1. Summary of test solution preparation and rationale for experiments addressing major ion toxicity of gold mill effluent to juvenile mysid shrimp.

Solution	Preparation	Rationale/remarks
effluent	stored at 4°C, in dark, no head space	not tested w/o modification, stable osmolality (= 12 ppt seawater) and stable pH (10) during storage
synthetic seawater	1. distilled water 2. salts in constant proportions to desired osmolality (see chapter 2)	diluent for all but one exposure solution
natural seawater	1. natural seawater 2. sand and 5 μm filtered 3. diluted to 12 ppt with distilled water	diluent for one exposure solution to test if effluent toxicity was specific to synthetic seawater formulation
pH adjusted effluent	1. effluent 2. HCl to pH 8.0 3. 12 ppt synthetic seawater diluent	range of dilutions to test effluent toxicity w/o pH influence, referred to as "effluent" throughout
osmolality and pH adjusted effluent (Chapter 2)	1. effluent 2. HCl to pH 8.0 3. synthetic seawater concentrate to osmolality of 31 ppt seawater 4. 31 ppt synthetic seawater diluent	range of dilutions to compare mysid shrimp to other species tested in chapter 2 w/o osmotic stress for marine species, referred to as "osmolality adjusted effluent" throughout

continued.

Table 1 continued.

Solution	Preparation	Rationale/remarks
simulated effluent	1. distilled water 2. salts to equal effluent major ion content 3. HCl to pH 8.0	tested at 37% and 100% to compare to finding of maximum toxicity at 37% effluent, and isolate toxicity resulting from major ion composition of effluent
Mg or Na adjusted effluent	1. 100% effluent 2. added MgCl or NaCl to = Mg or Na concentration in 12 ppt seawater	to test role of Mg and Na deficiencies in effluent toxicity
Ca or Mg adjusted simulated effluent	1. 37% simulated effluent 2. added less $CaCl_2$ and/or more MgCl than in simulated effluent to = Ca and/or Mg concentrations in 12 ppt seawater	to test role of excess Ca and Mg deficiency in effluent toxicity, 37% effluent was most toxic concentration

replicates of each exposure solution, each containing 5 animals, were prepared. Synthetic seawater (Bidwell and Spotte 1985), aged 1 to 2 wk, was used for experimental controls and for dilution (see Chapter 3, Table 2 for details of synthetic seawater preparation).

Effects of experimental exposures on the behavior of mysid shrimp were recorded on several occasions during the experiments. Chambers were rotated to create a slight current and carry affected animals to the center so they could be observed, after settling, in a single field of view under a dissecting microscope at 6.7x magnification. Mysid shrimp were not otherwise disturbed. After observing each chamber for 2 to 3 min, mysid shrimp were classified as: 1) mobile, if they changed their location, 2) immobile (did not change their location), but constantly moving their appendages, 3) immobile and sporadically moving their appendages, or 4) paralyzed, if no movement was exhibited. The criteria for an acceptable experiment was mobility in $\geq$ 90% of the control animals (WET test protocol states 90% survival, U.S. Environmental Protection Agency 1993a).

Determination of the concentration-response relationship for effluent

Toxicity of the effluent was determined in a 24 h experiment. Effluent was adjusted from pH 10.0 (pH of unadjusted effluent) to pH 8.0 by adding approximately 200 μL of 3 M HCl to 1 L of effluent. A nearly linear dilution series of 5 effluent concentrations plus a control was prepared using 12 ppt synthetic seawater. The osmolality of 12 ppt synthetic seawater matched the osmolality of effluent so

Table 2. Characteristics of the various test solutions used in experiments assessing major ion toxicity of gold mill effluent to juvenile mysid shrimp.

Variable	Effluent	Simulated effluent	Osmolality adjusted effluent[a]	Synthetic seawater
Osmolality (mmolal)	365	370	910	910
Salinity[b] (ppt)	12.6	12.8	31.0	31
Conductivity (mS)	21.1	20.7	36.3	35.5
Hardness (mg/L as $CaCO_3$)	11280	11000	13000	4200
Alkalinity (mg/L as $CaCO_3$)	31	<2	56	108
Bicarbonate (mg/L)	38	<0.001	68	44
Major ions (total dissolved, mg/L)				
Na^+	652	728	6078	8860
Ca^{2+}	5230	5660	4131	269
Mg^{2+}	13.8	13.8	410	914
K^+	254	248	462	433
Cl^-	9569	8960	20600	18000
SO_4^{2-}	1371	1380	1644	2511
Calculated molarity[c] (mM)	450	447	994	975

[a]Used only for toxicity tests in Chapter 3. Data were compared to results of current study.
[b]Converted from osmolality, expressed as the salinity of seawater at the measured osmolality.
[c]Sum of major ion molarities. Compare to measured osmolality.

that the osmolality of each effluent concentration was equal.

The toxicity of effluent to mysid shrimp in the above experiment was compared to the toxicity of the same effluent to mysid shrimp, except with addition of concentrated synthetic seawater to increase effluent osmolality to that of 31 ppt seawater (Chapter 3). The osmolality adjusted effluent was adjusted to pH 8.0 with 3 M HCl. A nearly linear dilution series of 5 osmolality adjusted effluent concentrations plus a control was prepared using 31 ppt synthetic seawater so that the osmolality of each exposure solution was equal. Refer to Chapter 3 for additional details on the toxicity tests using osmolality adjusted effluent.

The combined roles of major ions in effluent toxicity

To determine the combined role of major ions in effluent toxicity, the toxicities of effluent and simulated effluent were compared in a 10 h experiment. Simulated effluent was prepared to match the major ion composition of effluent (Table 2) using reagent grade salts as follows: 19.19 g $CaCl_2 2H_2O$; 2.028 g Na_2SO_4; 115.5 mg $MgCl_2 6H_2O$; 484.0 mg KCl; dissolved in distilled water to 1 L total. The pH was adjusted to 8.0 by adding 0.35 ml 0.1 M NaOH to 1 L of simulated effluent. The resulting osmolality of simulated effluent was nearly equal to the osmolality of actual effluent (Table 2). The simulated effluent experiment was concluded after 10 h because the toxicity of all exposures had become indistinguishable and the objective of the experiment had been met.

Only 37% and 100% effluent and simulated effluent were tested for determining the combined role of major ions in effluent toxicity. The objective was to determine if the finding of greater toxicity at 37% effluent than at 100% effluent (see Results) could be duplicated with simulated effluent. Effluent and simulated effluent were diluted to 37% with 12 ppt synthetic seawater. In addition, 37% effluent was prepared with natural seawater diluent rather than synthetic seawater for this experiment only. Natural seawater was used to determine if effluent toxicity was influenced by the synthetic seawater formulation. Natural seawater was obtained from 24 m depth in Auke Bay, Alaska, through the Juneau Center, School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, laboratory seawater system, passed through sand and 5 μm filters, and diluted with glass distilled water to 12 ppt.

The individual roles of major ions in effluent toxicity

To determine the individual roles of major ions in effluent toxicity, Ca^{2+}, Na^{+}, or Mg^{2+} concentrations were adjusted in 100% effluent or 37% simulated effluent. Ca^{2+} was selected for adjustment because it was in the greatest excess in effluent relative to seawater (Table 2). Na^{+} and Mg^{2+} were selected because they were the most deficient in effluent relative to seawater. Concentrations of 100% and 37% were used to investigate the reasons for the latter being more toxic (see Results). Simulated effluent was used to test for toxicity from excess Ca^{2+} since toxicity of the effluent and simulated effluent

were the same (see Results), and reduction of the Ca^{2+} concentration was easily achieved in the preparation of simulated effluent. More complicated methods would have been required to reduce the Ca^{2+} concentration in actual effluent. The experiment was conducted for 3 h because differences in toxicity of 37% and 100% effluent were greatest after 3 h (see Results).

Concentrations of Ca^{2+}, Na^{+}, or Mg^{2+} were adjusted in effluent or in simulated effluent to equal the concentrations of these ions in 12 ppt synthetic seawater (Table 3). Seawater at 12 ppt was used as a reference because the osmolality of the effluent was equal to that of 12 ppt seawater (note: adjusting the concentrations of Ca^{2+}, Na^{+}, or Mg^{2+} altered the osmolality of the solutions). Cl^{-} was selected as the counter ion because the amount of Cl^{-} associated with each cation adjustment was minor relative to the amount of Cl^{-} in effluent (Table 2) and in 12 ppt synthetic seawater. Solutions were adjusted to pH 8.0 by addition of 3 M NaOH or HCl.

Chemical analysis

The osmolality and pH of the stored effluent were measured throughout the study and remained nearly stable. Concentrations of total dissolved major ions, bicarbonate, hardness, and alkalinity were analyzed in the effluent, osmolality adjusted effluent, simulated effluent, and synthetic seawater (31 ppt) near the time of the effluent toxicity tests (10 to 12 mo after effluent production). Major ion concentrations in the solutions in which ion concentrations

Table 3. Preparation of solutions for testing the individual roles of major ions in gold mill effluent toxicity to mysid shrimp. All solutions were adjusted to pH 8.0. Ion concentrations were adjusted to equal the concentration in 12 ppt seawater (effluent osmolality was equal to 12 ppt seawater).

Solution	Preparation
37% simulated effluent	refer to Methods
37% simulated effluent with increased Mg^{2+}	3.02 g $MgCl_2 \cdot 6H_2O$/L added to simulated effluent rather than 115.5 mg
37% simulated effluent with decreased Ca^{2+}	389 mg $CaCl_2 \cdot 2H_2O$/L added to simulated effluent rather than 19.19 g
37% simulated effluent with increased Mg^{2+} and decreased Ca^{2+}	3.02 g $MgCl_2 \cdot 6H_2O$/L added to simulated effluent rather than 115.5 mg and 389 mg $CaCl_2 \cdot 2H_2O$/L rather than 19.19 g
100% effluent	refer to Methods
100% effluent with increased Mg^{2+}	added 2.90 g $MgCl_2 \cdot 6H_2O$ to effluent
100% effluent with increased Na^{+}	added 7.13 g NaCl to effluent

were adjusted were not confirmed analytically. Analyses of the effluent, osmolality adjusted effluent, and synthetic seawater were conducted by the U.S. Bureau of Mines, Spokane, Washington, USA. Due to later unavailability of the U.S. Bureau of Mines facility, simulated effluent analyses were conducted by a different laboratory (Montgomery Laboratories, Juneau, Alaska). Quality assurance between the two sets of analyses was based on close agreement of the nominal and measured ion concentrations in simulated effluent and in actual effluent. U.S. Environmental Protection Agency (1993b) methods were followed for analysis of: alkalinity (method 310.1), bicarbonate (method 310.1), Ca^{2+} (method 215.1), Cl^{-} (method 300.0), hardness (method 130.2), K^{+} (method 258.1), Mg^{2+} (method 242.1), Na^{+} (method 273.1) and SO_4^{2-} (method 300.0). Major ion concentrations of the diluted test solutions and 12 ppt synthetic seawater were calculated from the measured concentrations in the undiluted solutions.

Concentrations of individual major ions were converted to the proportion of the concentration in seawater at the osmolality of the exposure solution ([I]p):

$$[I]_p = A_e O_s / A_s O_e$$

where A_e is the total dissolved concentration of ion A in the exposure solution, O_s is the osmolality of seawater, A_s is the normal concentration of ion A in seawater, and O_e is the osmolality of the exposure solution. Using Na^{+} in effluent (Table 2) as an example:

$$[I]_p = (652 \text{ mg/L})(910 \text{ mmolal})/(8,860 \text{ mg/L})(365 \text{ mmolal}) = 0.183$$

that is, there was 0.183x as much Na^+ in the effluent as there would be in seawater if diluted to 365 mmolal. The roles of individual major ions in effluent toxicity were not apparent when ion concentrations were presented as mg/L.

Temperature, pH, and dissolved oxygen were measured after 1, 10 or 12, and 24 h in 1 replicate of each exposure solution using hand held meters. The pooled average (and standard deviation) temperature and dissolved oxygen concentration for all experiments were 19.8°C (0.4) and pH 7.70 (0.24). Dissolved oxygen did not range below 4.0 mg/L, in accordance with WET test protocol (U.S. Environmental Protection Agency 1993a). Osmolality was measured with a vapor pressure osmometer within 1 h of the start and at the end of each experiment. For all of the exposure solutions included in this study, osmolality ranged from 232 mmolal (= 7.9 ppt seawater) to 921 mmolal (= 31.5 ppt seawater).

Data analysis

A response index was created as a measure of toxicity. The response index differentiated toxicity in some solutions that would not have been differentiated had a single response been used. The response index incorporated the proportions of animals displaying one of three different responses into a single quantity. The response index (*RI*) was continuous between 0 and 1:

$$RI = (A/3 + B/2 + C)/n$$

where A is the number of animals in a chamber that were immobile (did not change their location) but constantly moving their appendages, B is the number that were immobile and sporadically moving their appendages, C is the number that were paralyzed, and n is the total number of animals in the chamber. The denominators for responses A, B, and C indicate the rank of response severity. The use and limitations of the response index were addressed in Chapter 3.

Response index values were based on proportions. As such, the arcsin transformation, $RI' = \arcsin(RI^{0.5})$, was used for statistical analyses to increase normality, homoscedasticity, and additivity of the response index (Zar 1996). Differences in RI' between effluent and simulated effluent and between solutions with adjusted concentrations of Ca^{2+}, Na^{+}, or Mg^{2+} were analyzed using single classification, model I ANOVA. When differences were significant ($p \leq 0.05$), Tukey's tests were conducted for pairwise comparisons. Untransformed means were reported.

Minimum detectable differences for RI were calculated as:

$$\delta = \sqrt{\frac{2ks^2\Phi^2}{n}}$$

where δ is the minimum detectable difference in untransformed response index units, k is the number of groups, s^2 is the mean square error

using raw data, n is the sample size, and Φ is a quantity incorporating k, n, and the probabilities of committing a Type I and Type II error (Zar 1996). Minimum detectable differences were based on $\alpha = 0.05$ and $1-\beta = 0.8$.

The relationship between toxicity and ion concentrations as $[I]_p$ for the combined data from all experiments was investigated using regression analysis (Zar 1996). It was hypothesized that an ion that was in excess relative to the seawater diluent was the source of toxicity and that an interaction between the ion that was in excess and a deficient ion was causing the decrease in toxicity at higher effluent concentrations. Separate regressions were conducted for each individual major ion. The hypothesized interaction between two ions was tested using the regression equation:

$$RI' = \alpha + \beta_1 X_1 + \beta_2 X_1 X_2 + \epsilon$$

where RI' is the transformed response index, α is the Y axis intercept, β is the slope, X_1 is an ion in excess as $[I]_p$, X_2 is a deficient ion as $[I]_p$, and ϵ is an error term. The $\log_{10}$ transformation was used for $[I]_p$. All statistical analyses were performed using SYSTAT 7.0 for Windows (SPSS Inc. 1997).

Results

Effluent toxicity

Maximum toxicity of effluent at osmolality equal to 12 ppt seawater (no addition of concentrated synthetic seawater) occurred at 37% effluent for all exposure durations < 24 h (Figure 1A, Appendix 12). This unusual finding of maximum toxicity at intermediate effluent concentrations was most pronounced after 3 h exposure, when toxicity decreased linearly from 37% to 100% effluent. Increasing the osmolality of the effluent to that of 31 ppt seawater by addition of concentrated synthetic seawater (Chapter 3) increased the rate of onset of toxicity at higher effluent concentrations and decreased the toxicity of 37% effluent for all exposure durations (Figure 1B). Toxicity generally stabilized by 2 h in osmolality adjusted effluent.

The combined roles of major ions in effluent toxicity

Toxicity was similar in effluent and simulated effluent (Figure 2, Appendix 12), indicating that effluent toxicity was attributable to one or more of the major ions that were included in simulated effluent. The toxicities of each 37% exposure were significantly greater than each 100% exposure after 1.5 and 3 h (Tukey's, $p \leq 0.05$). There were no significant differences in toxicity between 37% effluent and 37% simulated effluent or between 100% effluent and 100% simulated effluent. The use of natural seawater diluent did not significantly alter toxicity of 37% effluent. By 6 h, there were no significant differences in toxicity among any of the exposure solutions. The

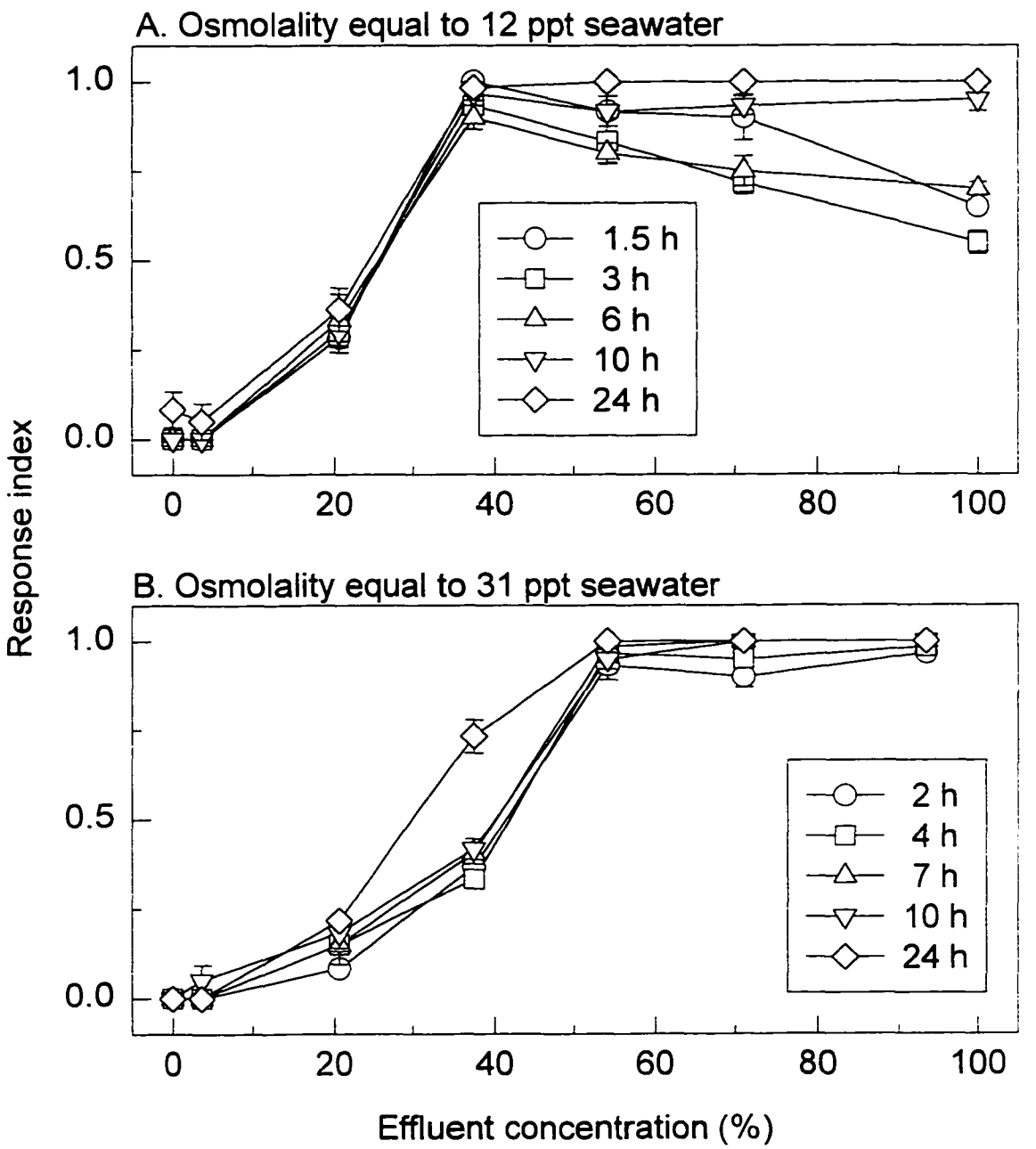

Figure 1. Toxicity of gold mill effluent to juvenile mysid shrimp. The response index incorporated the proportions of animals displaying different responses (0 = all animals mobile, not toxic, 1 = all animals paralyzed, most toxic). A. Effluent adjusted to pH 8.0, osmolality not adjusted and equal to 12 ppt seawater. Notice that maximum toxicity occurred at 37% effluent. B. Data from Chapter 2, effluent adjusted to pH 8.0 and osmolality adjusted to equal 31 ppt seawater. Notice the change in toxicity from A to B. Error bars are ± 1 standard error.

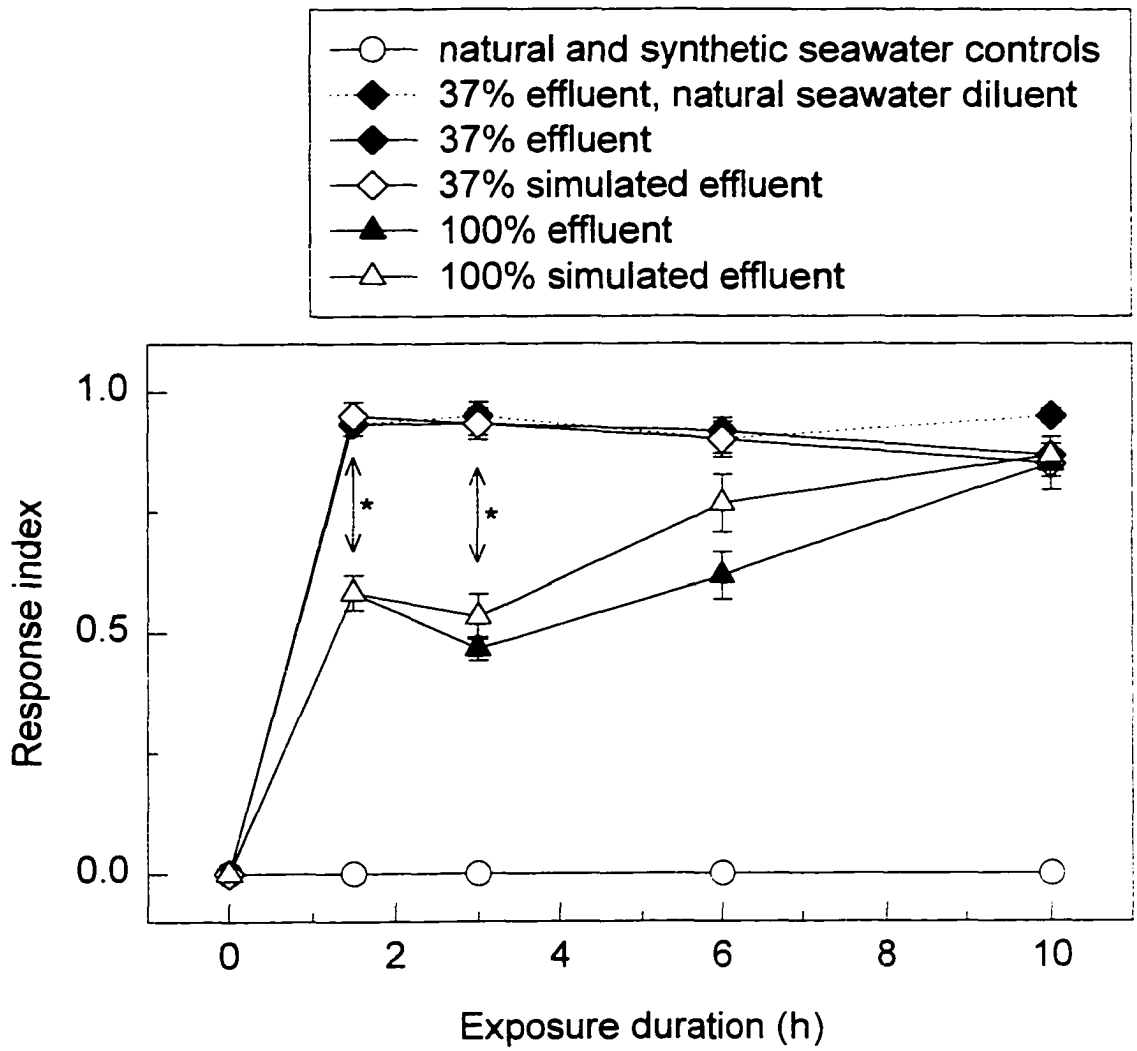

Figure 2. Comparison of gold mill effluent and simulated effluent toxicity to juvenile mysid shrimp. The response index incorporated the proportions of animals displaying different responses (0 = all animals mobile, not toxic, 1 = all animals paralyzed, most toxic). Simulated effluent contained only the major ions of effluent at the same concentrations as in effluent. Except where indicated, 37% solutions were prepared with 12 ppt synthetic seawater (equal to the osmolality of effluent). All solutions were adjusted to pH 8.0. Differences for each exposure duration among 37% and among 100% solutions were insignificant. Differences between 37% and 100% solutions (*) were significant after 1.5 and 3 h (Tukey's, $p \leq 0.05$). Notice that 37% concentrations were most toxic, as in Figure 1A. Error bars are ± 1 standard error.

minimum detectable difference in response index units was approximately 0.2 ($\alpha = 0.05$, $1-\beta = 0.8$).

The individual roles of major ions in effluent toxicity

An explanation for the finding of maximum effluent toxicity at 37% concentration was pursued to identify the source of effluent toxicity. Ca^{2+}, Na^{+}, and Mg^{2+} concentrations were adjusted in the effluent and simulated effluent to investigate if these ions contributed to effluent toxicity. Ca^{2+} was present in the effluent in the greatest excess, and Na^{+} and Mg^{2+} were the most deficient as $[I]_p$ (Table 4).

Reducing the concentration of Ca^{2+} in 37% simulated effluent significantly reduced toxicity (Figure 3, Appendix 12, Tukey's, $p \leq 0.05$). Since reducing the concentration of Ca^{2+} also reduced the osmolality of the solution, Ca^{2+} remained in excess in the adjusted solution ($[I]_p = 1.55$). Nonetheless, these results indicated that excess Ca^{2+} was probably the source of effluent toxicity. Cl^{-} was used as the counter ion for adjusting the concentration of Ca^{2+} and could not be eliminated as contributing to effluent toxicity. However, $[Cl]_p$ in 100% effluent was 1.33, whereas $[Ca]_p$ was 48. That is, Ca^{2+} was suspected to be toxic because of the magnitude of the difference in concentration in effluent relative to seawater.

Increasing the concentration of Na^{+} so that it was less deficient in effluent relative to seawater caused an increase in toxicity (Figure 3, Tukey's, $p \leq 0.05$), therefore, Na^{+} deficiency appeared to

Table 4. Concentrations of major ions relative to seawater and corrected for osmolality ($[I]_p$) for experiments assessing major ion toxicity of gold mill effluent to juvenile mysid shrimp. Concentrations are presented as the proportion of the concentration in seawater at the osmolality of effluent (= 12 ppt seawater) or at the osmolality of osmolality adjusted effluent[a] (= 31 ppt seawater). Notice the excess Ca^{2+}, the Na^{+} and Mg^{2+} deficiencies, and the moderating effect of osmolality adjustment on $[I]_p$.

	$[I]_p$	
Ion	effluent	osmolality adjusted effluent
Na^{+}	0.18	0.69
Ca^{2+}	48.43	15.36
Mg^{2+}	0.04	0.45
K^{+}	1.46	1.11
Cl^{-}	1.33	1.14
SO_4^{2-}	1.36	0.65

[a]Osmolality adjusted effluent was used only for tests in Chapter 3.

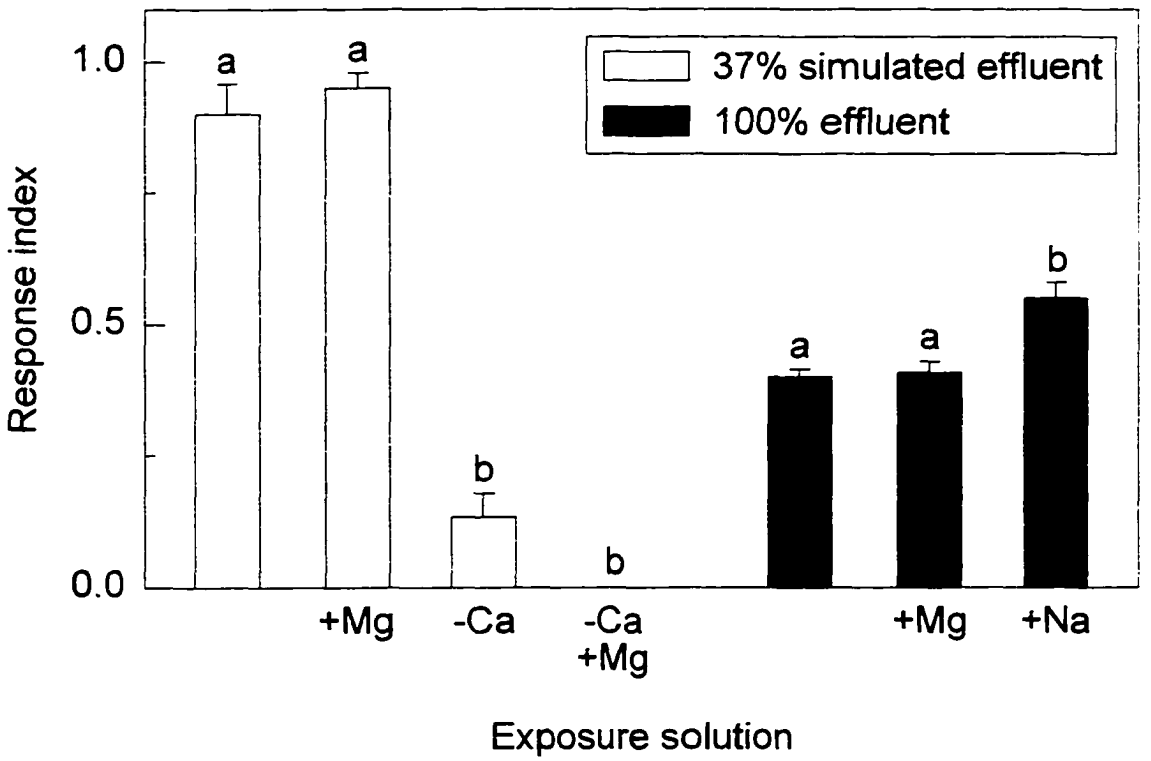

Figure 3. Effects of adjustment of Ca^{2+}, Na^{+}, and Mg^{2+} concentrations on toxicity of gold mill effluent and simulated effluent to juvenile mysid shrimp. The response index incorporated the proportions of animals displaying different responses (0 = all animals mobile, not toxic, 1 = all animals paralyzed, most toxic). All solutions were adjusted to pH 8. Signs (+ or -) preceding ions indicate the direction of the change in ion concentration relative to unadjusted solution. Ions concentrations were adjusted to equal the concentration in 12 ppt seawater (effluent osmolality was equal to 12 ppt seawater). Notice that toxicity decreased with decreased concentration of Ca^{2+} and toxicity increased with increased concentration of Na^{+}. In effluent, Ca^{2+} was in excess and Na^{+} was deficient relative to seawater. Bars of the same shade that do not share a letter were significantly different (Tukey's, $p \leq 0.05$). Error bars are $\pm$ 1 standard error.

decrease Ca^{2+} toxicity. Increasing the concentration of Mg^{2+} did not significantly affect toxicity of effluent or simulated effluent. The minimum detectable differences in response index units were 0.22 and 0.11 for the 37% and 100% solutions, respectively ($\alpha = 0.05$, $1-\beta = 0.8$).

To further test the roles of individual major ions in effluent toxicity, data from the effluent toxicity test (Figure 1A), osmolality adjusted effluent toxicity test (Figure 1B), simulated effluent experiment (Figure 2), and the Ca^{2+}, Na^{+}, Mg^{2+} adjustment experiment (Figure 3) were combined. Data from the 3 h observations were used because maximum discrimination in toxicity between exposures occurred after 3 h. For the osmolality adjusted effluent toxicity test, 2 h data were used because there was no 3 h observation.

Based on concentrations (mg/L) of major ions, an explanation for the finding of maximum effluent toxicity at 37% concentration was not apparent. When presented as the proportion of the ion concentration in seawater at the osmolality of the exposure solution ($[I]_p$), $[Ca]_p$ explained the greatest amount of the variability in toxicity (55%, Table 5). $[Cl]_p$, the counter ion for Ca^{2+} in the ion adjustment experiment, explained only 17% of the variation in toxicity. When the interaction of $[Ca]_p$ and $[Na]_p$ was included along with $[Ca]_p$, 76% of the variation in toxicity was explained. Including $[Ca]_p$ and the interaction of $[Ca]_p$ and $[Mg]_p$ explained 68% of the variation in toxicity, however, $[Mg]_p$ was ruled out as being related to toxicity based on the ion adjustment experiment (Figure 3).

Table 5. The proportion of the variation in toxicity (r^2) to juvenile mysid shrimp explained by major ions in gold mill effluent and solutions from related experiments. Only data from the 2 or 3 h observations were included. Ion concentrations were converted to $[I]_p$ for the analyses. Separate analyses were conducted for each single ion. Where Ca^{2+} and another ion are listed together, the model included Ca^{2+} and the interaction of Ca^{2+} and the other ion (refer to Methods). The model that included the interaction between 2 ions was tested only for Ca^{2+} and Na^+ or Mg^{2+}, based on results of the experiments. Slopes for all regressions were significantly different from zero ($p \leq 0.05$), with the exception of K^+.

Ions	r^2
Ca^{2+}	0.55
Na^+	0.10
Mg^{2+}	0.07
K^+	0.01
Cl^-	0.17
SO_4^{2-}	0.04
Ca^{2+}, Na^+	0.76
Ca^{2+}, Mg^{2+}	0.68

An assumption of regression analysis, that independent variables not be correlated (Zar 1996), was violated by testing for the $[Ca]_p$ and $[Na]_p$ interaction. For all experiments combined, the correlation coefficient (r) for $[Ca]_p$ and $[Na]_p$ was -0.98. That is, when $[Ca]_p$ was increased or decreased, the opposite occurred for $[Na]_p$. Nonetheless, when taken together with the individual experiments and with the separate regressions for each ion (Table 5), all results indicated that Ca^{2+} was the source of toxicity, and Na^+ deficiency reduced Ca^{2+} toxicity.

Discussion

Relation to whole effluent toxicity

Concentrations of many of the components of this gold mill effluent sample were not analyzed at the time of production. Effluent toxicity tests using mysid shrimp were also delayed. While it was conceivable that the effects of storage approximated some of the effects of effluent treatment at a mill site, this possibility was not investigated. As such, the results of this study should not be interpreted with respect to effluent as it leaves a gold mill. Rather, the results of this study should be interpreted as reflecting the toxicity of the conservative components of a sample of gold mill effluent. More importantly, this study served as an example of the complexities involved in assessing the toxicity of effluent with elevated TDS to marine organisms. In particular, this study provided information on Ca^{2+} toxicity, an ion that is often in excess in

effluents with elevated TDS (Price *et al.* 1990, Douglas and Horne 1997, Sauer *et al.* 1997).

Isolation of the Ca^{2+} and Na^{+} interaction

Several approaches were used to isolate Ca^{2+} as the source of effluent toxicity, and to identify a reduction in Ca^{2+} toxicity by Na^{+} deficiency. These approaches included comparing the magnitude of differences in the ionic composition of effluent and seawater, comparing effluent toxicity with and without osmolality adjustment, comparing toxicity of effluent and simulated effluent, adjusting concentrations of ion pairs, and combining these approaches to test for consistency. This weight of evidence approach was necessary due to correlations among several of the major ions, the possibility of toxicity from counter ions when adjusting selected ion concentrations, and the concurrent effect of ion adjustment on osmolality. Isolation of the interaction between Ca^{2+} and Na^{+} was aided by the unique concentration-response relationship of the effluent (Figure 1A).

The results of another study that used a different approach corroborate the results of the current study. Tolerance of *M. bahia* to excess Ca^{2+} after 96 h exposure was shown to increase with increasing salinity (Douglas and Horne 1997). Since salinity and the concentrations of Ca^{2+} and Na^{+} were all positively correlated, this finding was consistent with the current findings in that Ca^{2+} toxicity was dependent on the proportion of Ca^{2+} relative to Na^{+} and osmolality. Furthermore, the Douglas and Horne (1997) study indicated that the

interaction between Ca^{2+} and Na^{+} may persist through 96 h.

Possible physiological basis for a Ca^{2+} and Na^{+} interaction

M. bahia are euryhaline, maintaining slightly hyper- and hyposmotic hemolymph in seawater at less than and greater than 24 ppt, respectively (de Lisle and Roberts 1987). The gills are the main site of ion regulation in crustacea. Na^{+}-K^{+}-adenosine triphosphatase (Na^{+}-K^{+}-ATPase), located mainly in the gills, transports intracellular Na^{+} to the hemolymph in exchange for K^{+} and NH_4^{+}. The number of Na^{+} ions transported out of the cell exceeds the number of cations that are exchanged into the cell in a 3:2 ratio. This ratio creates an electrochemical gradient that drives exchange of external Na^{+} and Ca^{2+} for intracellular H^{+} (Towle 1993). An explanation for the Ca^{2+} and Na^{+} interaction, when viewed as proportions of the external salinity, could lie in the sharing of this transport mechanism by Ca^{2+} and Na^{+}.

A countering effect of Ca^{2+} and Na^{+} on Na^{+}-K^{+}-ATPase activity has been demonstrated. Maximum Na^{+}-K^{+}-ATPase activity occurred between 75 and 100 mM Na^{+} in the gills of three species of adult marine crabs (*Carcinus maenas, Cancer pagurus,* and *Macropipus puber*) and two species of freshwater crayfish (*Oronectes limosus* and *Astacus leptodactylus*) (Winkler 1986). Higher and lower Na^{+} concentrations inhibited Na^{+}-K^{+}-ATPase activity. Maximum Na^{+}-K^{+}-ATPase activity in *C. maenas* occurred in the absence of Ca^{2+} and decreased with increasing Ca^{2+} concentration. This study revealed the potential for Ca^{2+} and Na^{+} to exert opposite effects on Na^{+}-K^{+}-ATPase activity, depending on the

concentration of each ion (Winkler 1986).

This same relationship of Ca^{2+} and Na^{+} to Na^{+}-K^{+}-ATPase activity may exist for *M. bahia* since Winkler (1986) found similarities in this relationship among crustaceans that included freshwater, marine, stenohaline, and euryhaline species. Furthermore, whole-animal Na^{+}-K^{+}-ATPase activity in *M. bahia* has been shown to decrease in response to increased external concentrations of Ca^{2+} (Price *et al.* 1990), as demonstrated for *C. maenus* (Winkler 1986).

If maximum Na^{+}-K^{+}-ATPase activity in *M. bahia* occurs in the absence of Ca^{2+} (Price *et al.* 1990) and between 75 and 100 mM Na^{+}, as for other crustaceans (Winkler 1986), then Ca^{2+} and Na^{+} should have had a countering effect on Na^{+}-K^{+}-ATPase activity at intermediate effluent concentrations in the current study. The most toxic effluent concentration (37%) after 3 h contained 106 mM Na^{+}, near the concentration of Na^{+} that corresponds with maximum Na^{+}-K^{+}-ATPase activity in other species of crustaceans (Winkler 1986). This suggests that the effect of Na^{+} on Na^{+}-K^{+}-ATPase activity exceeded the opposite effect of Ca^{2+}, and that the maximum toxic effect of excess Ca^{2+} occurred when Na^{+}-K^{+}-ATPase activity was greatest. Based on this, a possible reason for Ca^{2+} toxicity is that Ca^{2+} influx into the gills, driven by the electrochemical gradient (Towle 1993), exceeded Na^{+} influx. The result was an intracellular Ca^{2+}:Na^{+} ratio that exceeded the range to which *M. bahia* are adapted.

Implications for other toxicity test species

The same effluent sample used for the current study was used to compare the sensitivity of larval sheepshead minnows (*Cyprinodon variegatus*), another common WET test species, and larvae of species that are indigenous to the southern coast of Alaska: Pacific herring (*Clupea harengus pallasi*); red king crab (*Paralithodes camtschaticus*); northern shrimp (*Pandalus borealis*) (Chapter 3). Sublethal sensitivities of these crustaceans and mysid shrimp, to osmolality adjusted effluent was greater than sublethal sensitivities of the two species of fish. The most sensitive of these species was mysid shrimp. Given the similarity of the response of Na^+-K^+-ATPase in several species of crustaceans to Ca^{2+} and Na^+ (Winkler 1986, Price *et al.* 1990), an interaction between Ca^{2+} and Na^+ is also likely to have occurred for the other species of crustaceans tested in Chapter 3.

Pertinent information on Na^+-K^+-ATPase in Pacific herring was not located. The lack of sensitivity of sheepshead minnows was probably due to high tolerance to excess Ca^{2+}, relative to mysid shrimp. The 96-h LC50 for sheepshead minnows for Ca^{2+} is 4,970 mg/L, compared to a 48-h LC50 of 570 mg/L Ca^{2+} for *M. bahia* (Price 1989). Since the Ca^{2+} concentration of the highest concentration of osmolality adjusted effluent was 4,131 mg/L (Table 2), Ca^{2+} concentrations in the effluent were probably sub-toxic to sheepshead minnows after 24 h.

Conclusions

Excess Ca^{2+} in this sample of gold mill effluent was the source of toxicity to mysid shrimp. Na^{+} deficiency in the effluent, relative to seawater, decreased toxicity resulting from excess Ca^{2+}. This study demonstrated the importance of understanding major ion toxicity when interpreting results of toxicity studies using effluent with elevated concentrations of TDS.

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Literature Cited

Altayaran, A.M. and I.M. Madany. 1992. Impact of a desalination plant on the physical and chemical properties of seawater, Bahrain. *Wat. Res.* 26:435-441.

Bidwell, J.P. and S. Spotte. 1985. *Artificial Seawaters: Formulas and Methods*. Jones and Bartlett Publ., Boston, MA, USA, 349 pp.

Burgess, R.M., K.T. Ho, M.D. Tagliabue, A. Kuhn, R. Comeleo, P. Comeleo, G. Modica, and G.E. Morrison. 1995. Toxicity characterization of an industrial and a municipal effluent discharging to the marine environment. *Mar. Poll. Bull.* 30:524-535.

de Lisle, P.F. and M.H. Roberts, Jr. 1987. Osmoregulation in the estuarine mysid, *Mysidopsis bahia* Molenock: comparison with other mysid species. *Comp. Biochem. Physiol.* 88A:369-372.

Douglas, W.S. and M.T. Horne. 1997. The interactive effects of essential ions and salinity on the survival of *Mysidopsis bahia* in 96-h acute toxicity tests of effluents discharged to marine and estuarine receiving waters. *Environ. Toxicol. Chem.* 16:1996-2001.

Florida Department of Environmental Protection. 1995. Protocols for determining major-seawater-ion toxicity in membrane-technology water-treatment concentrate. Bureau of Laboratories.

Ho, K.T., K. Mitchell, M. Zappala, and R.M. Burgess. 1995. Effects of brine addition on effluent toxicity and marine toxicity identification evaluation (TIE) manipulations. *Environ. Toxicol. Chem.* 14:245-249.

Ho, K. and D. Caudle. 1997. Ion toxicity and produced water. *Environ. Toxicol. Chem.* 16:1993-1995.

Ingersoll, C.G., F.J. Dwyer, S.A. Burch, M.K. Nelson, D.R. Buckler, and J.B. Hunn. 1992. The use of freshwater and saltwater animals to distinguish between the toxic effects of salinity and contaminants in irrigation drain water. *Environ. Toxicol. Chem.* 11:503-511.

Mendez, R., J.M. Lema, and M. Soto. 1995. Treatment of seafood-processing wastewaters in mesophilic and thermophilic anaerobic filters. *Water Environment Research* 67:33-45.

Meyer, J.S., D.A. Sanchez, J.A. Brookman, D.B. McWhorter, and H.L. Bergman. 1985. Chemistry and aquatic toxicity of raw oil shale leachates from Piceance Basin, Colorado. *Environ. Toxicol. Chem.* 4:559-572.

Price, E.E. 1989. Response of freshwater and saltwater toxicity test species to calcium and salinity concentrations encountered in toxicity tests. Ph.D. thesis, University of North Texas, Order number 9016203.

Price, E.E., M.J. Donahue, K.L. Dickson, and J.H. Rodgers, Jr. 1990. Effects of elevated calcium concentration on Na-K-ATPase activity of two euryhaline species, *Cyprinodon variegatus* and *Mysidopsis bahia*. *Bull. Environ. Contam. Toxicol.* 44:121-128.

Sauer, T.C., H.J. Costa, J.S. Brown, and T.J. Ward. 1997. Toxicity identification evaluations of produced-water effluents. *Environ. Toxicol. Chem.* 16:2020-2028.

SPSS, Inc. 1997. SYSTAT 7.0. Chicago, IL, USA,

Towle, D.W. 1993. Ion transport systems in membrane vesicles isolated from crustacean tissues. *J. Exper. Zool.* 265:387-396.

U.S. Environmental Protection Agency. 1991. Methods for aquatic toxicity identification evaluations: Phase I toxicity characterization procedures. EPA/600/6-91/003. Washington, DC, USA.

U.S. Environmental Protection Agency. 1993a. Methods for measuring the acute toxicity of effluents to freshwater and marine organisms. 4th ed., EPA/600/4-90/027F. Washington, DC, USA.

U.S. Environmental Protection Agency. 1993b. Methods for the determination of inorganic substances in environmental samples. EPA/600/R-93/100. Washington, DC, USA.

Winkler, A. 1986. Effects of inorganic sea water constituents on branchial Na-K-ATPase activity in the shore crab *Carcinus maenus*. *Mar. Biol.* 92:537-544.

Zar, J.H. 1996. *Biostatistical Analysis* 3rd ed., Prentice-Hall, Upper Saddle River, NJ, USA, 662 pp.

Chapter 5

General Conclusions

This thesis provided methods and data that should assist in assessing or predicting environmental impacts of any coastal, metal mining operation. The effluent toxicity studies were applicable to an operation that treats and discharges some or all of its process water. Due to brief exposure durations in the ocean, an effluent would have to be highly toxic to present a realistic risk of acute toxicity to planktonic organisms. Despite a low risk of acute toxicity in the ocean, effluent toxicity tests are used to monitor effluent toxicity and to provide standards for dischargers. If the sensitivity of reference species relative to indigenous species varies from site to site, different dischargers may be held to different standards. The sensitivity to effluent of the two reference species that were studied bracketed that of the indigenous species, providing some context for the Kensington Mine. The species sensitivity comparisons were dependent on the response, illustrating a limitation of acute toxicity studies that include only one response.

It is important to limit interpretation of the effluent toxicity studies to conservative effluent components since it was necessary to store the effluent for several months. The source of effluent toxicity was Ca^{2+}. An important implication of this finding is that effluent toxicity was not the result of cyanide, other milling

reagents, or heavy metals, as may be suspected. Another important implication is that a direct marine discharge, rather than a freshwater discharge, would minimize the risk of toxicity since Ca^{2+} is abundant in the ocean. The latter implication could have been based on measurement of TDS alone. An interactive effect of Na^{+} on Ca^{2+} toxicity illustrated the need to consider interactions when building models to predict major ion toxicity.

The tailings colonization study demonstrated that tailings are not necessarily toxic. Macrofauna were selected for study, in part, because no other component of the ecosystem would be more intimately associated with tailings after STD and because macrofauna are an important food source for larger animals. However, recovery of a food source does not guarantee that predators will return and prosper after STD. Studies on feeding and growth by predators of macrofauna would be the logical, next experiment.

Aversion to what may appear to be an "out of sight, out of mind" solution may lead some to view STD unfavorably. However, the alternatives should be weighed before forming an opinion. In addition to the long-term risk of metal contamination and erosion, terrestrial tailings disposal may permanently alter the landscape, a habitat type, or the course of a stream. In comparison, STD may cause only temporary impacts to a widespread, relatively uniform habitat. The challenge is to consider application of STD where appropriate and to accurately predict the site specific impacts so that informed regulatory decisions can be made.

Appendix 1. Location and depth of STD candidate site samples from Lynn Canal.

Transect	Latitude (o N)	Longitude (o W)	Depth (m)
shallow	58.833	135.151	188
shallow	58.841	135.166	175
shallow	58.873	135.175	188
shallow	58.878	135.162	198
shallow	58.890	135.168	187
deep	58.833	135.218	304
deep	58.842	135.214	301
deep	58.864	135.216	309
deep	58.874	135.216	309
deep	58.887	135.212	308
deep	58.825	135.214	305

Appendix 2. Whole assemblage attributes for the colonization experiment. Means and 95% confidence limits (CL), p values (p) from ANOVA, pairwise comparisons (Tukey's), R, reference; T, tailings; A, ambient, and minimum detectable differences (mdd) as percent of the reference mean (alpha = 0.05, 1-beta = 0.8). Data are from are from Figure 6, Chapter 2.

		Reference		Tailings		Ambient			Pairwise comparisons			
Attribute	Sampling period	Mean	95% CL	Mean	95% CL	Mean	95% CL	p	R-T	R-A	T-A	mdd
abundance	1	730.1	593.7 880.6	622.1	541.5 708.3	456.7	355.7 570.3	0.002	0.338	0.002	0.052	32.3
	2	641.1	563.9 723.3	485.5	399.6 579.7	297.6	228.5 375.8	0.000	0.029	0.000	0.001	26.3
	3	1047.5	921.9 1181.3	898.8	807.6 994.9	918.8	803.8 1041.4	0.102	-	-	-	22.7
biomass	1	100.6	80.7 125.5	71.2	59.8 84.8	109.7	68.8 174.8	0.085	-	-	-	57.9
	2	206.4	144.3 295.3	190.4	155.7 233.0	130.1	99.0 171.0	0.034	0.894	0.039	0.101	45.5
	3	388.2	335.4 449.4	373.0	306.4 454.0	145.3	109.6 192.6	0.000	0.952	0.000	0.000	30.0
number of taxa	1	43.1	39.9 46.4	42.5	39.6 45.5	37.4	33.9 41.0	0.016	0.959	0.023	0.044	15.7
	2	40.0	36.5 43.6	39.1	35.1 43.4	27.1	22.9 31.6	0.000	0.947	0.000	0.000	21.0
	3	47.7	43.0 52.6	49.7	45.9 53.6	39.4	36.7 42.2	0.000	0.710	0.004	0.001	17.1
biomass/ abundance	1	0.140	0.107 0.183	0.115	0.090 0.145	0.244	0.180 0.331	0.000	0.487	0.007	0.000	77.7
	2	0.310	0.221 0.435	0.393	0.303 0.509	0.422	0.279 0.640	0.339	-	-	-	84.0
	3	0.367	0.310 0.434	0.414	0.363 0.472	0.154	0.115 0.206	0.000	0.636	0.000	0.000	28.7

Appendix 3. Identity of macrofauna in samples from the colonization experiment and Lynn Canal samples. Taxa code corresponds to Appendices 4 and 5. Information is ordered alphabetically by phylum, then by class within phylum, and so on. Where letters (a, b, c ...) are listed for species, the taxon was not identified to species but was distinguishable from other taxa.

Taxa code	Phylum	Class	Order	Family	Genus	Species
1	Annelida	Polychaeta	-	-	-	a
2	Annelida	Polychaeta	Capitellida	Arenicolidae	-	a
3	Annelida	Polychaeta	Capitellida	Capitellidae	-	a
4	Annelida	Polychaeta	Capitellida	Capitellidae	Decamastus	gracilis
5*	Annelida	Polychaeta	Capitellida	Capitellidae	Mediomastus	californiensis
6	Annelida	Polychaeta	Capitellida	Maldanidae	-	a
7	Annelida	Polychaeta	Capitellida	Maldanidae	-	b
8	Annelida	Polychaeta	Capitellida	Maldanidae	Axiothella	a
9	Annelida	Polychaeta	Capitellida	Maldanidae	Clymenella	torquata
10	Annelida	Polychaeta	Capitellida	Maldanidae	Isocirrus	longiceps
11	Annelida	Polychaeta	Capitellida	Maldanidae	Notoproctus	pacificus
12	Annelida	Polychaeta	Capitellida	Maldanidae	Petaloproctus	tenuis
13	Annelida	Polychaeta	Capitellida	Maldanidae	Praxillella	gracilis
14	Annelida	Polychaeta	Capitellida	Maldanidae	Praxillella	praetermissa
15	Annelida	Polychaeta	Chaetopterida	Chaetopteridae	Spiochaetopterus	costarum
16	Annelida	Polychaeta	Cirratulida	Cirratulidae	-	a
17	Annelida	Polychaeta	Cirratulida	Cirratulidae	-	b
18	Annelida	Polychaeta	Cirratulida	Cirratulidae	-	c
19	Annelida	Polychaeta	Cirratulida	Cirratulidae	Chaetozone	cf. setosa
20	Annelida	Polychaeta	Cirratulida	Cirratulidae	Cirriformia	spirabranchia
21	Annelida	Polychaeta	Cossurida	Cossuridae	Cossura	a
22	Annelida	Polychaeta	Cossurida	Cossuridae	Cossura	soyeri
23	Annelida	Polychaeta	Eunicida	Lumbrinereidae	Lumbrineris	luti
24	Annelida	Polychaeta	Eunicida	Lumbrinereidae	Lumbrineris	similabris
25	Annelida	Polychaeta	Flabelligerida	Flabelligeridae	-	a
26	Annelida	Polychaeta	Flabelligerida	Flabelligeridae	Brada	villosa
27	Annelida	Polychaeta	Flabelligerida	Flabelligeridae	Pherusa	plumosa
28	Annelida	Polychaeta	Magelonida	Magelonidae	Magelona	berkeleyi

Appendix 3 continued.

Taxa code	Phylum	Class	Order
29	Annelida	Polychaeta	Opheliida
30	Annelida	Polychaeta	Opheliida
31	Annelida	Polychaeta	Opheliida
32	Annelida	Polychaeta	Opheliida
33	Annelida	Polychaeta	Orbiniida
34	Annelida	Polychaeta	Orbiniida
35	Annelida	Polychaeta	Orbiniida
36	Annelida	Polychaeta	Orbiniida
37	Annelida	Polychaeta	Orbiniida
38*	Annelida	Polychaeta	Oweniida
39	Annelida	Polychaeta	Oweniida
40	Annelida	Polychaeta	Phyllodocida
41	Annelida	Polychaeta	Phyllodocida
42	Annelida	Polychaeta	Phyllodocida
43	Annelida	Polychaeta	Phyllodocida
44	Annelida	Polychaeta	Phyllodocida
45	Annelida	Polychaeta	Phyllodocida
46	Annelida	Polychaeta	Phyllodocida
47	Annelida	Polychaeta	Phyllodocida
48	Annelida	Polychaeta	Phyllodocida
49	Annelida	Polychaeta	Phyllodocida
50	Annelida	Polychaeta	Phyllodocida
51	Annelida	Polychaeta	Phyllodocida
52	Annelida	Polychaeta	Phyllodocida
53	Annelida	Polychaeta	Phyllodocida
54	Annelida	Polychaeta	Phyllodocida
55	Annelida	Polychaeta	Phyllodocida
56	Annelida	Polychaeta	Phyllodocida
57	Annelida	Polychaeta	Phyllodocida
58	Annelida	Polychaeta	Phyllodocida
59	Annelida	Polychaeta	Phyllodocida

Family	Genus	Species
Opheliidae	Ophelina	a
Opheliidae	Ophelina	acuminata
Opheliidae	Ophelina	breviata
Scalibregmatidae	Scalibregma	inflatum
Orbiniidae	Leitoscoloplos	panamensis
Paraonidae	-	a
Paraonidae	Aricidea	a
Paraonidae	Levinsenia	gracilis
Paraonidae	Paraonella	a
Oweniidae	Galathowenia	oculata
Oweniidae	Owenia	fusiformis
Glyceridae	Hemipodus	borealis
Goniadidae	Glycinde	picta
Goniadidae	Glycinde	polygnatha
Goniadidae	Goniada	brunnea
Hesionidae	-	a
Nephtyidae	Nephtys	cf. cornuta
Nephtyidae	Nephtys	ciliata
Nephtyidae	Nephtys	cornuta
Nereidae	Nereis	pelagica
Pholodidae	Pholoe	glabra
Phyllodocidae	Eteone	longa
Phyllodocidae	Eteone	tuberculata
Phyllodocidae	Phyllodoce	a
Polynoidae	-	a
Polynoidae	Enoe	senta
Polynoidae	Hesperonoe	a
Polynoidae	Lepidonotus	squamatus
Polynoidae	Polynoe	canadensis
Sphaerodoridae	Sphaerodoropsis	minuta
Sphaerodoridae	Sphaerodoropsis	sphaerulifer

Appendix 3 continued.

Taxa code	Phylum	Class	Order
60	Annelida	Polychaeta	Phyllodocida
61	Annelida	Polychaeta	Phyllodocida
62	Annelida	Polychaeta	Phyllodocida
63	Annelida	Polychaeta	Phyllodocida
64	Annelida	Polychaeta	Sabellida
65	Annelida	Polychaeta	Sabellida
66	Annelida	Polychaeta	Sabellida
67	Annelida	Polychaeta	Sabellida
68	Annelida	Polychaeta	Sabellida
69	Annelida	Polychaeta	Sabellida
70	Annelida	Polychaeta	Sabellida
71	Annelida	Polychaeta	Sabellida
72	Annelida	Polychaeta	Sabellida
73	Annelida	Polychaeta	Sabellida
74	Annelida	Polychaeta	Spionida
75	Annelida	Polychaeta	Spionida
76	Annelida	Polychaeta	Spionida
77	Annelida	Polychaeta	Spionida
78	Annelida	Polychaeta	Spionida
79	Annelida	Polychaeta	Spionida
80	Annelida	Polychaeta	Spionida
81	Annelida	Polychaeta	Spionida
82	Annelida	Polychaeta	Spionida
83	Annelida	Polychaeta	Spionida
84	Annelida	Polychaeta	Spionida
85	Annelida	Polychaeta	Sternapsida
86	Annelida	Polychaeta	Terebellida
87	Annelida	Polychaeta	Terebellida
88	Annelida	Polychaeta	Terebellida
89*	Annelida	Polychaeta	Terebellida
90	Annelida	Polychaeta	Terebellida

Family	Genus	Species
Syllidae	-	a
Syllidae	Brania	a
Syllidae	Micropodarke	dubia
Syllidae	Syllis (Typosyllis)	alternata
Sabellidae	-	a
Sabellidae	-	b
Sabellidae	Chone	a
Sabellidae	Chone	duneri
Sabellidae	Chone	mollis
Sabellidae	Euchone	analis
Sabellidae	Euchone	incolor
Sabellidae	Laonome	kroyeri
Sabellidae	Myxicola	infundibulum
Sabellidae	Sabella (Demonax)	media
Spionidae	-	a
Spionidae	Dipolydora	cardalia
Spionidae	Dipolydora	caulleryi
Spionidae	Dipolydora	socialis
Spionidae	Polydora	a
Spionidae	Prionospio	a
Spionidae	Prionospio	b
Spionidae	Prionospio	steenstrupi
Spionidae	Spio	butleri
Spionidae	Spio	cirrifera
Spionidae	Spio	filicornis
Sternapsidae	Sternapsis	scutata
Ampharetidae	Ampharete	acutifrons
Ampharetidae	Amphicteis	a
Ampharetidae	Amphicteis	mucronota
Ampharetidae	Amphicteis	scaphobranchiata
Ampharetidae	Amphisamytha	bioculata

Appendix 3 continued.

Taxa code	Phylum	Class	Order
91*	Annelida	Polychaeta	Terebellida
92	Annelida	Polychaeta	Terebellida
93	Annelida	Polychaeta	Terebellida
94	Annelida	Polychaeta	Terebellida
95	Annelida	Polychaeta	Terebellida
96*	Annelida	Polychaeta	Terebellida
97	Annelida	Polychaeta	Terebellida
98*	Annelida	Polychaeta	Terebellida
99	Arthropoda	Malacostraca	Amphipoda
100	Arthropoda	Malacostraca	Amphipoda
101	Arthropoda	Malacostraca	Amphipoda
102	Arthropoda	Malacostraca	Amphipoda
103	Arthropoda	Malacostraca	Amphipoda
104	Arthropoda	Malacostraca	Amphipoda
105	Arthropoda	Malacostraca	Amphipoda
106	Arthropoda	Malacostraca	Amphipoda
107	Arthropoda	Malacostraca	Amphipoda
108	Arthropoda	Malacostraca	Amphipoda
109	Arthropoda	Malacostraca	Amphipoda
110	Arthropoda	Malacostraca	Amphipoda
111	Arthropoda	Malacostraca	Amphipoda
112	Arthropoda	Malacostraca	Cumacea
113	Arthropoda	Malacostraca	Cumacea
114	Arthropoda	Malacostraca	Cumacea
115	Arthropoda	Malacostraca	Cumacea
116	Arthropoda	Malacostraca	Cumacea
117	Arthropoda	Malacostraca	Cumacea
118	Arthropoda	Malacostraca	Cumacea
119	Arthropoda	Malacostraca	Cumacea
120	Arthropoda	Malacostraca	Cumacea
121	Arthropoda	Malacostraca	Cumacea

Family	Genus	Species
Ampharetidae	Lysippe	a
Pectinariidae	Pectinaria	granulata
Terebellidae	-	a
Terebellidae	Artacama	conifera
Terebellidae	Pista	brevibranchiata
Terebellidae	Polycirrus	a
Trichobranchidae	Terebellides	a
Trichobranchidae	Terebellides	stroemi
-	-	a
-	-	b
Ampeliscidae	Byblis	veleronis
Atylidae	Atylus	a
Caprellidae	Caprella	alaskana
Isaeidae	Protomedeia	articulta
Lysianassidae	Anonyx	cf. laticoxae
Oedicerotidae	Monoculodes	a
Pardaliscidae	Pardalisca	tenuipes
Phoxocephalidae	Eobrolgus	spinosus
Phoxocephalidae	Rhepoxynius	vigitegus
Pontogeneiidae	Paramoera	cf. serrata
Synopiidae	Syrrhoe	longifrons
-	-	a
-	-	b
-	-	c
-	-	d
-	-	e
-	-	f
-	-	g
Diastylidae	Diastylis	a
Lampropidae	-	a
Leuconiidae	Edorella	a

Appendix 3 continued.

Taxa code	Phylum	Class	Order
122	Arthropoda	Malacostraca	Cumacea
123	Arthropoda	Malacostraca	Decapoda
124	Arthropoda	Malacostraca	Isopoda
125	Arthropoda	Ostracoda	-
126	Arthropoda	Ostracoda	-
127	Arthropoda	Ostracoda	-
128	Arthropoda	Pycnogonida	-
129	Echindodermata	Holothuroidea	Dendrochirotida
130	Echindodermata	Holothuroidea	Dendrochirotida
131	Echindodermata	Ophiuroidea	Ophiurida
132	Echindodermata	Ophiuroidea	Ophiurida
133	Echiura	-	Echiuroinea
134	Mollusca	Aplacophora	Chaetodermatida
135	Mollusca	Bivalvia	-
136	Mollusca	Bivalvia	Myoida
137	Mollusca	Bivalvia	Mytiloida
138	Mollusca	Bivalvia	Nuculoida
139*	Mollusca	Bivalvia	Nuculoida
140	Mollusca	Bivalvia	Nuculoida
141	Mollusca	Bivalvia	Nuculoida
142	Mollusca	Bivalvia	Pholadomyoida
143*	Mollusca	Bivalvia	Veneroida
144*	Mollusca	Bivalvia	Veneroida
145	Mollusca	Bivalvia	Veneroida
146	Mollusca	Bivalvia	Veneroida
147	Mollusca	Bivalvia	Veneroida
148*	Mollusca	Gastropoda	Archaeogastropoda
149*	Mollusca	Gastropoda	Cephalaspidea
150	Mollusca	Gastropoda	Cephalaspidea
151	Mollusca	Gastropoda	Gymnosomata
152	Mollusca	Gastropoda	Mesogastropoda

Family	Genus	Species
Leuconiidae	Leucon	a
Pinnixidae	Pinnixa	eburnea
Pleurogoniidae	Pleurogonium	rubicundum
-	-	a
-	-	b
-	-	c
Phoxichilidiidae	-	a
Phyllophoridae	-	a
Sclerodactylidae	Eupentacta	pseudoquinquesemita
Ophiuridae	-	a
Ophiuridae	Ophiura	a
Echiuridae	Echiurus	echiurus alaskensis
-	-	a
-	-	a
Hiatellidae	Hiatella	arctica
Mytilidae	Musculus	discors
Nuculanidae	Nuculana	fossa
Nuculanidae	Nuculana	minuta
Nuculidae	Nucula	tenuis
Yoldidae	Yoldia	scissuratella
Pandoridae	Pandora	filosa
Cardiidae	Clinocardium	ciliatum
Cardiidae	Serripes	groenlandicus
Carditidae	Cyclocardia	ovatum
Tellinidae	Tellina	a
Thyasiridae	Axiopsida	serricata
Trochidae	Solariella	vancouverensis
Cylichnidae	Cylichna	a
Cylichnidae	Cylichna	alba
Clionidae	Clione	limacina
Rissoidae	Alvania	compacta

Appendix 3 continued.

Taxa code	Phylum	Class	Order	Family	Genus	Species
153	Mollusca	Gastropoda	Mesogastropoda	Trichotropidae	Trichotropis	cancellata
154*	Mollusca	Gastropoda	Neogastropoda	Muricidae	Scabrotrophon	maltzani
155	Mollusca	Gastropoda	Neogastropoda	Neptunidae	-	a
156	Mollusca	Gastropoda	Neogastropoda	Turridae	Oenopota	excurvata
157	Mollusca	Gastropoda	Pyramidellacea	Pyramidellidae	Turbonilla	a
158	Mollusca	Gastropoda	Thecosomata	Limacinidae	Limacina	helicina
159*	Mollusca	Scaphopoda	Dentaliida	Dentaliidae	Rhabdus	rectius
160	Nematoda	-	-	-	-	a
161	Nematoda	-	-	-	-	b
162	Nemertea	Anopla	Heteronemertea	Lineidae	Lineus	a
163	Phoronida	-	-	Phoronidae	*Phoronopsis*	harmeri
164	Priapulida	-	Priapulomorpha	Priapulidae	Priapulus	caudatus
165	Sipunucula	-	Sipunuculida	Golfingiidae	Golfingia	pugettensis
166	unknown	-	-	-	-	a
167	unknown	-	-	-	-	b

* Identity confirmed by L. Harris, Curator of polychaetes, Los Angeles County Museum, Los Angeles, CA, USA (polychaeta) or T. Rice, Curator of the Sea and Shore Museum, Port Gamble, WA, USA (mollusca).

Appendix 4. Total abundance by taxon of the combined replicates for each sampling period and sample type for the colonization experiment and the Lynn Canal samples (Ref., reference sediment; Tail., tailings; Amb., ambient). Sample size = 10 for colonization experiment samples, 5 for 187 m Lynn Canal samples, and 6 for 306 m Lynn Canal samples. Taxa code corresponds to Appendices 3 and 5. Information is ordered alphabetically by phylum, then by class within phylum, and so on (see Appendix 3).

	Sampling period 1			Sampling period 2			Sampling period 3			Lynn Canal	
Taxa code	Ref.	Tail.	Amb.	Ref.	Tail.	Amb.	Ref.	Tail.	Amb.	187 m	306 m
1	0	0	1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	20	0	0	0	0	0
3	1	0	1	0	0	0	4	20	0	0	0
4	13	1	222	5	15	191	311	163	288	0	0
5	1,869	1,162	683	1,948	905	546	2,382	1,627	942	3	0
6	0	0	1	0	0	0	0	0	0	2	0
7	0	0	0	0	0	0	0	0	0	1	0
8	0	0	9	3	0	23	73	9	14	0	0
9	77	97	18	401	207	64	187	210	34	0	0
10	0	0	0	0	0	1	0	0	3	0	0
11	0	0	0	0	0	0	0	0	0	3	0
12	0	0	0	0	2	18	0	0	0	0	0
13	0	0	6	1	0	2	1	0	2	0	0
14	0	1	1	1	0	0	1	1	2	0	0
15	6	3	4	9	15	1	3	7	1	0	0
16	0	0	0	0	0	0	0	0	0	2	2
17	0	0	0	0	0	0	0	0	0	1	0
18	0	0	0	0	0	0	0	0	0	1	2
19	5	4	5	9	1	3	8	10	1	0	0
20	1	3	102	3	5	101	17	16	138	0	0
21	0	0	0	0	0	0	0	0	0	76	53
22	2	0	147	8	1	130	25	1	202	0	0
23	106	69	467	131	58	494	345	266	701	0	0

Appendix 4 continued.

Taxa code	Sampling period 1			Sampling period 2			Sampling period 3			Lynn Canal	
	Ref.	Tail.	Amb.	Ref.	Tail.	Amb.	Ref.	Tail.	Amb.	187 m	306 m
24	0	0	0	0	0	0	0	0	0	15	1
25	2	0	0	0	0	0	0	0	0	0	0
26	0	1	0	1	3	0	0	0	0	0	0
27	38	62	3	14	6	0	2	13	4	0	0
28	128	121	87	94	81	29	126	133	65	0	0
29	24	79	0	0	0	0	0	0	0	0	0
30	401	280	17	88	26	5	93	82	11	0	0
31	54	53	5	0	0	0	18	25	3	0	0
32	1	0	2	1	0	0	1	2	1	0	0
33	57	27	20	17	11	17	10	5	22	0	0
34	0	0	0	0	0	0	0	0	0	4	5
35	3	1	11	5	1	1	2	1	13	4	1
36	0	0	0	0	0	0	0	0	0	98	25
37	2	0	10	3	0	4	1	4	15	0	0
38	1,074	1,286	480	1,213	1,310	132	1,876	1,983	632	21	0
39	37	28	9	36	24	1	25	44	3	0	0
40	2	2	1	0	2	0	0	0	0	0	0
41	38	29	35	5	3	7	62	40	53	0	0
42	278	285	111	101	90	46	182	166	150	0	0
43	0	0	0	0	0	0	0	0	0	0	1
44	0	0	0	0	0	0	3	0	1	0	0
45	0	0	0	0	0	0	0	0	0	77	39
46	3	8	7	4	8	1	2	9	5	0	0
47	387	233	440	420	406	218	640	317	1,100	0	0
48	77	48	53	42	30	34	81	78	94	0	0
49	639	643	437	549	450	118	512	363	672	0	0
50	55	33	37	36	17	50	81	50	101	8	0
51	0	3	2	0	0	0	0	0	0	0	0

Appendix 4 continued.

Taxa code	Sampling period 1			Sampling period 2			Sampling period 3			Lynn Canal	
	Ref.	Tail.	Amb.	Ref.	Tail.	Amb.	Ref.	Tail.	Amb.	187 m	306 m
52	0	0	1	0	0	0	0	2	0	0	0
53	0	0	0	0	0	1	0	0	0	0	0
54	13	19	7	0	0	0	25	28	0	0	0
55	6	5	1	0	0	0	1	1	0	0	0
56	9	1	10	0	0	0	16	3	1	0	0
57	0	0	0	0	0	0	1	2	0	0	0
58	0	2	3	0	0	0	5	10	8	0	0
59	5	2	5	3	1	3	2	1	3	0	0
60	0	0	0	0	1	0	0	0	0	0	0
61	1	1	0	1	1	0	0	2	4	0	0
62	0	0	0	0	0	0	0	0	1	0	0
63	0	0	1	0	0	0	2	0	2	0	0
64	1	0	0	0	1	0	1	1	0	0	0
65	8	2	0	1	0	1	4	2	0	0	0
66	0	0	0	0	0	0	0	1	0	0	0
67	7	9	2	16	23	1	15	29	1	0	0
68	1	0	0	0	2	0	1	2	0	0	0
69	2	8	0	12	11	0	2	2	0	0	0
70	0	1	0	0	2	0	2	2	3	0	0
71	83	83	0	49	81	0	46	97	0	0	0
72	3	6	0	5	11	0	1	16	0	0	0
73	8	1	0	2	0	0	31	22	0	0	0
74	0	0	0	0	0	0	0	0	0	2	1
75	1	5	0	0	14	0	0	0	0	0	0
76	0	0	0	0	3	0	0	0	0	0	0
77	71	78	3	30	67	17	48	51	3	0	0
78	0	0	0	0	0	0	0	0	0	1	0
79	0	0	0	2	0	1	1	0	0	0	0

Appendix 4 continued.

Taxa code	Sampling period 1			Sampling period 2			Sampling period 3			Lynn Canal	
	Ref.	Tail.	Amb.	Ref.	Tail.	Amb.	Ref.	Tail.	Amb.	187 m	306 m
80	2	0	0	0	1	0	0	0	0	0	0
81	581	573	618	265	193	378	1,926	1,822	2,245	0	0
82	1	1	0	0	0	0	0	0	0	0	0
83	20	3	0	5	4	1	6	11	0	0	0
84	0	0	0	0	0	0	0	0	1	0	0
85	0	0	0	0	0	0	0	0	0	0	10
86	0	0	0	0	0	0	5	1	0	0	0
87	0	0	0	0	0	0	0	0	0	1	0
88	1	1	0	1	0	0	1	0	0	0	0
89	4	8	0	12	6	0	13	21	1	0	0
90	2	5	3	0	0	1	1	0	0	0	0
91	119	105	41	145	94	18	144	94	23	5	0
92	15	3	5	0	0	1	2	3	1	0	0
93	0	0	0	0	0	0	3	0	0	0	0
94	38	19	0	3	0	0	2	2	0	0	0
95	5	0	0	0	0	0	1	0	0	0	0
96	129	45	2	7	11	0	43	62	0	0	0
97	2	4	0	0	0	0	5	4	0	0	0
98	102	56	2	179	174	4	250	239	5	0	0
99	8	2	1	0	2	0	6	19	0	3	0
100	0	0	0	0	0	0	0	0	0	3	0
101	0	6	2	10	9	0	2	2	1	0	0
102	9	1	1	2	4	2	31	6	0	0	0
103	1	3	1	0	0	0	3	2	0	0	0
104	316	264	14	112	97	4	65	94	13	0	0
105	0	6	0	3	7	2	41	8	3	0	0
106	3	1	1	0	2	0	3	9	7	0	0
107	2	1	1	9	2	0	4	6	1	0	0

Appendix 4 continued.

Taxa code	Sampling period 1			Sampling period 2			Sampling period 3			Lynn Canal	
	Ref.	Tail.	Amb.	Ref.	Tail.	Amb.	Ref.	Tail.	Amb.	187 m	306 m
108	6	3	0	0	0	0	1	7	0	0	0
109	0	0	1	0	0	0	0	0	0	0	0
110	0	1	1	0	0	0	2	0	0	0	0
111	0	0	0	0	0	0	1	1	0	0	0
112	0	0	0	0	0	0	0	0	0	3	0
113	0	0	1	2	0	0	1	1	1	11	0
114	0	0	0	0	0	0	0	1	0	7	1
115	0	0	6	0	0	1	0	0	6	3	0
116	0	0	1	0	2	0	0	0	0	0	0
117	0	0	0	0	0	0	0	0	0	11	0
118	0	0	0	0	8	0	9	3	1	12	2
119	1	0	0	0	1	2	0	0	1	0	0
120	1	0	1	0	0	1	0	1	1	0	0
121	4	8	0	11	11	2	14	10	0	0	0
122	6	11	4	14	46	8	11	24	5	0	0
123	1	0	1	0	0	1	1	1	3	0	0
124	1	1	2	1	1	0	0	2	5	0	0
125	9	4	14	3	4	3	4	4	65	0	0
126	48	38	52	8	6	2	13	31	92	1	0
127	0	0	1	0	0	0	0	0	0	0	0
128	1	0	0	0	0	0	0	0	0	0	0
129	8	4	10	5	2	6	3	7	19	0	0
130	0	0	3	0	0	0	0	0	3	0	0
131	0	0	0	0	0	0	0	0	1	0	0
132	1	2	2	3	9	1	4	6	8	0	0
133	2	1	0	1	1	0	4	0	0	0	0
134	0	0	2	0	0	0	0	0	1	2	2
135	0	0	0	0	0	0	0	0	0	0	5

Appendix 4 continued.

Taxa code	Sampling period 1			Sampling period 2			Sampling period 3			Lynn Canal	
	Ref.	Tail.	Amb.	Ref.	Tail.	Amb.	Ref.	Tail.	Amb.	187 m	306 m
136	1	2	4	1	3	2	7	8	1	0	0
137	0	2	0	3	0	0	0	0	0	0	0
138	5	8	10	8	4	0	3	2	18	0	0
139	3	2	0	6	4	1	0	6	1	1	0
140	3	7	10	20	60	6	46	38	30	1	3
141	1	0	1	2	1	0	7	12	1	0	0
142	0	0	0	0	0	0	0	0	0	0	1
143	5	5	1	6	10	0	4	5	2	0	0
144	16	12	0	13	7	0	5	3	2	0	0
145	4	0	1	0	1	0	3	0	1	0	0
146	17	27	9	15	22	9	43	42	35	0	0
147	119	93	75	110	59	8	160	226	353	3	5
148	0	0	0	0	1	0	0	0	1	0	0
149	0	0	0	0	0	0	0	0	0	0	1
150	1	1	0	5	10	0	0	1	0	0	0
151	0	0	0	0	0	0	1	0	0	0	0
152	1	3	2	1	15	0	8	9	0	0	0
153	0	0	0	0	1	0	0	1	0	0	0
154	0	0	0	0	0	1	0	0	0	0	0
155	0	1	0	0	0	0	1	0	1	0	0
156	0	0	3	0	0	4	1	4	8	0	1
157	0	0	1	1	0	2	0	0	0	0	0
158	0	0	0	0	0	0	0	0	0	0	7
159	0	0	0	0	0	0	0	0	0	4	6
160	63	50	199	63	30	241	103	70	724	8	0
161	0	0	0	0	0	0	0	0	0	1	0
162	35	15	47	44	30	44	88	113	73	0	0
163	66	33	29	47	30	11	105	58	28	0	0

Appendix 4 continued.

Taxa code	Sampling period 1			Sampling period 2			Sampling period 3			Lynn Canal	
	Ref.	Tail.	Amb.	Ref.	Tail.	Amb.	Ref.	Tail.	Amb.	187 m	306 m
164	0	0	0	0	0	0	0	0	0	0	5
165	1	4	6	35	45	5	56	2	150	0	0
166	0	0	0	0	0	0	0	0	0	1	0
167	0	0	1	0	0	0	0	0	0	0	0

Appendix 5. Taxonomic authority and functional grouping of macrofauna in samples from the colonization experiment and Lynn Canal samples (subdepo, subsurface deposit; surfdepo, surface deposit; carn, carnivore; susp, suspension). Taxa code corresponds to Appendices 3 and 4. Information is ordered alphabetically by phylum, then by class within phylum, and so on (see Appendix 3). Refer to Appendix 6 for references.

Taxa code	Authority	Feeding type	Sediment association type	Reference
1	-	-	-	-
2	-	-	burrower	Wells 1959
3	-	subdepo	burrower	Barnes 1987
4	Hartman 1963	-	burrower	Barnes 1987
5	Hartman 1944	subdepo	burrower	Barnes 1987 Kalke and Montagna 1991 Lastra et al. 1991
6	-	subdepo	tube builder	Kudenov 1977 Levin et al. 1995
7	-	subdepo	tube builder	same as taxa code 6
8	Johnson 1901	subdepo	tube builder	same as taxa code 6
9	Leidy 1855	subdepo	tube builder	Kudenov 1977 Fauchald and Jumars 1979
10	Moore 1908	subdepo	tube builder	same as taxa code 6
11	Moore 1906	subdepo	tube builder	same as taxa code 6
12	Theel 1879	surfdepo	tube builder	same as taxa code 6
13	M. Sars 1861	surfdepo	tube builder	same as taxa code 6
14	Malmgren 1866	surfdepo	tube builder	same as taxa code 6
15	Claparede 1870	suspension	tube builder	Fauchald and Jumars 1979 Sendall et al. 1995
16	-	surfdepo	-	Fauchald and Jumars 1979
17	-	surfdepo	-	Fauchald and Jumars 1979
18	-	surfdepo	-	Fauchald and Jumars 1979
19	Malmgren 1867	surfdepo	-	Fauchald and Jumars 1979
20	Moore 1904	surfdepo	-	Fauchald and Jumars 1979
21	-	surfdepo	-	Blake 1993

Appendix 5 continued.

Taxa code	Authority	Feeding type
22	Laubier 1961	surfdepo
23	Berkeley & Berkeley 1944	surfdepo/carn
24	Treadwell 1926	surfdepo/carn
25	-	surfdepo
26	Rathke 1843	surfdepo
27	Muller 1776	surfdepo
28	-	surfdepo/susp
29	-	subdepo
30	Orsted 1843	subdepo
31	-	subdepo
32	Rathke 1843	surfdepo
33	Monroe 1933	subdepo
34	-	surfdepo
35	-	surfdepo
36	Tauber 1879	surfdepo
37	Hobson 1972	surfdepo
38	Zachs 1923	surfdepo/susp
39	delle Chiaje 1841	surfdepo/susp
40	Johnson 1901	carnivore
41	E.Berkeley 1927	carnivore
42	Hartman 1925	carnivore
43	Treadwell 1906	carnivore

Sediment association type	Reference
-	Blake 1993
burrower	Carrasco and Oyarzun 1988
	Yun and Kikuchi 1989
	Netto and Da Cunha Lana 1994
burrower	same as taxa code 23
burrower	same as taxa code 9
burrower	same as taxa code 9
burrower	same as taxa code 9
burrower	same as taxa code 9
burrower	Fauchald and Jumars 1979
	Riser 1987
	Tamaki 1987
burrower	same as taxa code 29
burrower	same as taxa code 29
-	Blake 1993
burrower	same as taxa code 9
	Blake 1993
burrower	Fauchald and Jumars 1979
	Gaston et al. 1992
burrower	same as taxa code 34
burrower	same as taxa code 34
burrower	same as taxa code 34
tube builder	Fauchald and Jumars 1979
	Gambi 1987
	Dauvin and Thiebaut 1994
tube builder	same as taxa code 38
burrower	same as taxa code 9
-	Fauchald and Jumars 1979
burrower	same as taxa code 9
-	Fauchald and Jumars 1979

Appendix 5 continued.

Taxa code	Authority	Feeding type
44	-	herbivore
45	Berkeley & Berkeley 1945	carnivore
46	Muller 1776	carnivore
47	Berkeley & Berkeley 1944	carnivore
48	Linnaeus 1761	surfdepo
49	Hartman 1944	carnivore
50	Fabricius 1780	carnivore
51	-	carnivore
52	-	carnivore
53	-	-
54	Moore 1902	carnivore
55	-	carnivore
56	Linnaeus 1767	carnivore
57	McIntosh 1874	carnivore
58	Webster & Benedict 1887	surfdepo
59	Moore 1909	surfdepo
60	-	-
61	-	-
62	Hessle 1925	-
63	Moore 1908	-
64	-	suspension
65	-	suspension
66	-	suspension
67	Malmgren 1867	suspension
68	Bush 1904	suspension
69	Kroyer 1856	suspension
70	Hartman 1965	suspension

Sediment association type	Reference
surface	same as taxa code 9
burrower	Fauchald and Jumars 1979
surface	Fauchald and Jumars 1979
	McDermott 1987
burrower	Fauchald and Jumars 1979
burrower	Barnes 1987
	Olivier et al. 1995
surface	Fauchald and Jumars 1979
surface	same as taxa code 9
surface	same as taxa code 9
surface	same as taxa code 9
surface	Barnes 1987
surface	same as taxa code 9
surface	same as taxa code 9
surface	same as taxa code 9
surface	same as taxa code 9
-	Fauchald and Jumars 1979
-	Fauchald and Jumars 1979
surface	Barnes 1987
surface	Barnes 1987
surface	Barnes 1987
surface	Barnes 1987
tube builder	Barnes 1987
tube builder	same as taxa code 9
tube builder	same as taxon 68
tube builder	Fauchald and Jumars 1979
	Yun and Kikuchi 1989
tube builder	same as taxa code 67
tube builder	same as taxa code 9
tube builder	same as taxa code 9

Appendix 5 continued.

Taxa code	Authority	Feeding type
71	Malmgren 1866	suspension
72	Renier 1804	suspension
73	Bush 1904	suspension
74	-	-
75	Berkeley 1927	-
76	Mesnil 1897	-
77	Schmarda 1861	-
78	-	-
79	-	surfdepo
80	-	surfdepo
81	Malmgren 1867	surfdepo
82	-	surfdepo/susp
83	Banse & Hobson 1968	surfdepo/susp
84	-	surfdepo/susp
85	Renier 1807	-
86	Grube 1860	surfdepo
87	-	subdepo
88	Moore 1923	subdepo
89	Moore 1906	subdepo
90	Moore 1906	surfdepo
91	Malmgren 1866	surfdepo
92	Linnaeus 1767	subdepo
93	-	surfdepo
94	Moore 1906	surfdepo

Sediment association type	Reference
tube builder	same as taxa code 9
tube builder	same as taxa code 9
tube builder	same as taxa code 9
tube builder	Barnes 1987
tube builder	Barnes 1987
tube builder	Barnes 1987
tube builder	Barnes 1987
tube builder	Barnes 1987
tube builder	Barnes 1987
	Noji and Noji 1991
	Platell and Potter 1996
tube builder	same as taxa code 79
tube builder	same as taxa code 79
tube builder	Bock and Miller 1996a, b
tube builder	Bock and Miller 1996a, b
tube builder	Bock and Miller 1996a, b
-	-
tube builder	Dales 1963
	Bock and Miller 1996a
tube builder	Dales 1963
tube builder	Komar and Taghon 1985
	Dales 1963
	Penry 1989
tube builder	same as taxa code 88
tube builder	same as taxa code 9
tube builder	same as taxa code 9
tube builder	same as taxa code 9
	Vavelle and Grasset 1990
tube builder	Fauchald and Jumars 1979
-	Fauchald and Jumars 1979

Appendix 5 continued.

Taxa code	Authority	Feeding type
95	Moore 1923	surfdepo
96	-	surfdepo
97		surfdepo
98	M. Sars 1835	surfdepo
99	-	-
100	-	-
101	Barnard 1954	suspension
102	-	-
103	Mayer 1903	-
104	Barnard 1962	-
105	Gurjanova 1962	scavenger
106	-	carnivore
107	G.O. Sars 1893	carnivore
108	Holmes 1905	-
109	Barnard 1971	-
110	-	herbivore
111	Shoemaker 1964	-
112	-	-
113	-	-
114	-	-
115	-	-
116	-	-
117	-	-
118	-	-
119	-	-
120	-	-
121	-	-
122	-	-
123	Wells 1928	-

Sediment association type	Reference
tube builder	same as taxa code 86
tube builder	Fauchald and Jumars 1979
tube builder	Fauchald and Jumars 1979
tube builder	Fauchald and Jumars 1979
-	-
-	-
tube builder	Dauvin and Bellan-Santini 1990
surface	Barnes 1987
surface	Barnes 1987
-	-
burrower	Charmasson and Calmet 1987 Bousfield and Staude 1994
burrower	Beare and Moore 1994
surface	Kaartvedt et al. 1994
burrower	Lenihan et al. 1995
burrower	Lenihan et al. 1995
surface	Brawley and Fei 1987
-	-
burrower	Jones 1976, Barnes 1987
burrower	Jones 1976, Barnes 1987
burrower	Jones 1976, Barnes 1987
burrower	Jones 1976, Barnes 1987
burrower	Jones 1976, Barnes 1987
burrower	Jones 1976, Barnes 1987
burrower	Jones 1976, Barnes 1987
burrower	Jones 1976, Barnes 1987
burrower	Jones 1976, Barnes 1987
burrower	Jones 1976, Barnes 1987
burrower	Jones 1976, Barnes 1987
-	-

Appendix 5 continued.

Taxa code	Authority	Feeding type
124	G.O.Sars 1863	surfdepo
125	-	-
126	-	-
127	-	-
128	-	-
129	-	surfdepo/susp
130	Deichmann 1938	surfdepo/susp
131	-	surfdepo/carn
132	-	surfdepo/carn
133	-	surfdepo
134	-	-
135	-	surfdepo
136	Linnaeus 1767	surfdepo
137	Linnaeus 1767	surfdepo
138	Baird 1863	surfdepo
139	Fabricius 1776	surfdepo
140	Montagu 1808	surfdepo
141	-	surfdepo
142	Carpenter 1864	surfdepo
143	Fabricius 1780	surfdepo
144	Bruguiere 1789	surfdepo
145	-	surfdepo
146	-	surfdepo
147	Carpenter 1864	surfdepo
148	E.A. Smith 1887	-
149	-	-
150	Carpenter 1864	-
151	Phipps 1774	-
152	Carpenter 1864	-

Sediment association type	Reference
surface	Barnes 1987
surface	Barnes 1987
surface	Barnes 1987
surface	Barnes 1987
surface	Schram and Hedgpeth 1978
surface	Smith 1983
surface	Smith 1983
surface	Jangoux and Lawrence 1982 Sokolova et al. 1995
surface	same as taxa code 131
burrower	Nickell and Atkinson 1994
-	-
burrower	Stanley 1975, Morton 1983
burrower	Stanley 1975, Morton 1983
burrower	Stanley 1975, Morton 1983
burrower	Stanley 1975, Morton 1983
burrower	Stanley 1975, Morton 1983
burrower	Stanley 1975, Morton 1983
burrower	Stanley 1975, Morton 1983
burrower	Stanley 1975, Morton 1983
burrower	Stanley 1975, Morton 1983
burrower	Stanley 1975, Morton 1983
burrower	Stanley 1975, Morton 1983
burrower	Stanley 1975, Morton 1983
burrower	Stanley 1975, Morton 1983
surface	Gainey 1976
surface	Gainey 1976
surface	Gainey 1976
surface	Gainey 1976
surface	Gainey 1976

Appendix 5 continued.

Taxa code	Authority	Feeding type	Sediment association type	Reference
153	Hinds 1849	-	surface	Gainey 1976
154	Dall 1919	-	surface	Gainey 1976
155	-	-	surface	Gainey 1976
156	Carpenter 1864	-	surface	Gainey 1976
157	-	-	surface	Gainey 1976
158	Phipps 1774	-	surface	Gainey 1976
159	Carpenter 1864	subdepo	burrower	Gainey 1972, Bilyard 1974
160	-	-	burrower	Croll and Matthews 1977
161	-	-	burrower	Croll and Matthews 1977
162	-	carnivore	burrower	Turbeville and Ruppert 1983 McDermott and Roe 1985
163	Pixell 1912	suspension	tube builder	Emig 1982
164	Lamarck 1816	carnivore	burrower	Barnes 1987, Shirley 1990
165	Fisher 1952	subdepo	burrower	Kohn and Rice 1971
166	-	-	-	-
167	-	-	-	-

Appendix 6. References for classification of taxa by feeding type and sediment association type in Appendix 5.

Barnes, R.D. 1987. *Invertebrate Zoology*. 5th ed., Saunders College Publishing, Philadelphia, PA, USA. 893 pp.

Beare, D.J. and P.G. Moore. 1994. Observations on the biology of a rare British marine amphipod: *Monoculodes gibbosus* (Crustacea: Amphipoda: Oedicerotidae). *J. Mar. Biol. Assoc. U.K.* 74:193-201.

Bilyard, G.R. 1974. The feeding habits and ecology of *Dentalium entale stimpsoni*. *Veliger* 17:126-138.

Blake, J.A. 1993. Life history analysis of five dominant infaunal polychaete species from the continental slope off North Carolina. *J. Mar. Biol. Assoc. U.K.* 73:123-141.

Bock, M.J. and D.C. Miller. 1996a. Fluid flow and suspended particulates as determinants of polychaete feeding behavior. *J. Mar. Res.* 54:565-588.

Bock, M.J. and D.C. Miller. 1996b. Particle-bound organic matter as a cue for suspension feeding in polychaetes. *Proceedings*, 24th Annual Benthic Ecology Meeting, Columbia, SC, USA, March 7-10, 1996, p. 20.

Bousfield, E.L. and C.P. Staude. 1994. The impact of J.L. Barnard on North American Pacific amphipod research: A tribute. *Amphipacifica* 1:3-16.

Brawley, S.H. and X.G. Fei. 1987. Studies of mesoherbivory in aquaria and in an unbarricaded mariculture farm on the Chinese coast. *J. Phycol*. 23:614-623.

Carrasco, F.D. and C. Oyarzun. 1988. Diet of the polychaete *Lembrineris tetraura* (Schmarda) (Lumbrineridae) in a polluted softbottom environment. *Bull. Mar. Sci*. 42:358-365.

Charmasson, S.S. and D.P. Calmet. 1987. Distribution of scavenging Lysianassidae amphipods *Eurythenes gryllus* in the Northeast Atlantic: comparison with studies held in the Pacific. *Deep Sea Res. A Oceanogr. Res. Pap.* 34:1509-1523.

Croll, N.A. and B.E. Matthews. 1977. *Biology of Nematodes*. John Wiley and Sons, New York, USA. 201 pp.

Dales, R.P. 1963. *Annelids*. Hutchinson University Library, London, England.

Appendix 6 continued.

Dauvin, J.C. and D. Bellan-Santini. 1990. An overview of the amphipod genus *Haploops* (Ampeliscidae). *J. Mar. Biol. Assoc. U.K.* 70:887-903.

Dauvin, J.C. and E. Thiebaut. 1994. Is *Owenia fusifcrmis* Delle Chiaje a cosmopolitan species? *Proceedings*, 4th International Polychaete Conference, J.C. Dauvin, L. Laubier and D.J. Reish, eds., Angers, France, July 27-August 1, 1992, 162:383-404.

Emig, C.C. 1982. The biology of Phoronida. *Adv. Mar. Biol.* 19:1-89.

Fauchald, K. and P.A. Jumars. 1979. The diet of worms: a study of polychaete feeding guilds. *Oceanogr. Mar. Biol. Ann. Rev.* 17:193-284.

Gainey, L.F. 1972. The use of the foot and captacula in the feeding of *Dentalium*. *Veliger* 15:29-34.

Gainey, L.F. 1976. Locomotion in the Gastropoda: functional morphology of the foot in *Neritina reclivata* and *Thais rustica*. *Malacologia* 15:411-431.

Gambi, M.C. 1987. Functional morphology and feeding behavior of *Owenia fusiformis* in relation to the environment. *Oebalia,* Congr. della Societa Italiana di Biologia Marina, Naples, Italy, September 24-28, 1987, pp. 145-155.

Gaston, G.R., J.A. McLelland, and R.W. Heard. 1992. Feeding biology, distribution, and ecology of two species of benthic polychaetes: *Paraonis fulgens* and *Paraonis pygoenigmatica* (Polychaeta: Paraonidae). *Gulf Res. Rep.* 8:395-400.

Jangoux, M. and J.M. Lawrence (eds.). 1982. *Echinoderm Nutrition*. A.A. Balkema, Rotterdam. 654 pp.

Jones, N.S. 1976. British cumaceans. *Synopses of the British fauna No. 7. Linn. Soc. London*. Academic Press, London, England. 62 pp.

Kaartvedt, S., C.L. Van Dover, L.S. Mullineaux, P.H. Wiebe, and S.M. Bollens. 1994. Amphipods on a deep-sea hydrothermal treadmill. *Deep Sea Res. A Oceanogr. Res. Pap.* 41:179-195.

Kalke, R.D. and P.A. Montagna. 1991. The effect of freshwater inflow on macrobenthos in the Lavaca River delta and upper Lavaca Bay, Texas. *Contrib. Mar. Sci.* 32:49-71.

Appendix 6 continued.

Kohn, A.J. and M.E. Rice. 1971. Biology of Sipuncula and Echiura. *Bioscience* 21:583-584.

Komar, P.D. and G.L. Taghon. 1985. Analyses of the settling velocities of fecal pellets from the subtidal polychaete *Amphicteis scaphobranchiata*. *J. Mar. Res.* 43:605-614.

Kudenov, J.D. 1977. The functional morphology of feeding in three species of maldanid polychaetes. *Zool. J. Linn. Soc.* 60:95-109.

Lastra, M., J.M. Parada, A. Sanchez, and J. Mora. 1991. Temporal evolution of an *Abra abra* community affected by harbor dredging activities in Santander Bay (northern Spain). *Thalassas* 9:87-97.

Lenihan, H.S., K.A. Kiest, K.E. Conlan, P.N. Slattery, B.H. Konar, and J.S. Oliver. 1995. Patterns of survival and behavior in Antarctic benthic invertebrates exposed to contaminated sediments: field and laboratory bioassay experiments. *J. Exp. Mar. Biol. Ecol.* 192:233-255.

Levin, L., N. Blair, G. Plaia, D. DeMaster, C. Martin, and W. Fornes. 1995. Maldanid polychaetes as vehicles for rapid subduction of phytodetritus on the North Carolina slope. *Proceedings*, 23rd Benthic Ecology Meeting, J.P. Grassle, A. Kelsey E. Oates and P.V. Snelgrove, eds., New Brunswick, NJ, USA, March 17-19, 1995.

McDermott, J.J. 1987. The distribution and food habits of *Nephtys bucera* Ehlers, 1868, (Polychaeta: Nephtyidae) in the surf zone of a sandy beach. *Proc. Biol. Soc. Wash.* 100:21-27.

McDermott, J.J. and P. Roe. 1985. Food, feeding behavior and feeding ecology of nemerteans. *Amer. Zool.* 25:113-126.

Morton, B. 1983. Feeding and digestion in Bivalvia. *The Mollusca*, Vol. 5, Pt. 2. K.M. Wilbur, ed., Academic Press, NY, USA. pp. 65-147.

Netto, S.A. and P. Da Cunha Lana. 1994. Effects of sediment disturbance on the structure of benthic fauna in a subtropical tidal creek of southeastern Brazil. *Mar. Ecol. Prog. Ser.* 106:239-247.

Nickell, L.A. and R.J.A. Atkinson. 1994. Observations on the behavior of *Thalassema thalassemum* (Echiura: Echiuridae). *J. Mar. Biol. Assoc. U.K.* 74:963-966.

Appendix 6 continued.

Noji, C.I.M. and T.T. Noji. 1991. Tube lawns of spionid polychaetes and their significance for recolonization of disturbed benthic substrates. *Meeresforschung Rep. Mar. Res.* 33:235-246.

Olivier, M., G. Desrosiers, A. Caron, C. Retiere, and A. Caillou. 1995. Selective responses of the polychaetes *Nereis diversicolor* (O.F. Mueller) and *Nereis virens* (Sars) to alimentary stimulation: utilization of algae and halophytes. *Can. J. Zool. Rev. Can. Zool.* 73:2307-2317.

Penry, D.L. 1989. Tests of kinematic models for deposit-feeders' guts: Patterns of sediment processing by *Parastichopus californicus* (Stimpson) (Holothuroidea) and *Amphicteis scaphobranchiata* Moore (Polychaeta). *J. Exp. Mar. Biol. Ecol.* 128:127-146.

Platell, M.E. and I.C. Potter. 1996. Influence of water depth, season, habitat and estuary location on the macrobenthic fauna of a seasonally closed estuary. *J. Mar. Biol. Assoc. U.K.* 76:1-21.

Riser, N.W. 1987. Observations on the genus *Ophelia* (Polychaeta: Opheliidae) with the description of a new species. *Ophelia* 28:11-29.

Schram, F.R. and J.W. Hedgpeth. 1978. Locomotory mechanisms in Antarctic pycnogonids. *Zool. Jour. Linn. Soc.* 63:145-169.

Sendall, K.A., A.R. Fontaine, and D. O'Foighil. 1995. Tube morphology and activity patterns related to feeding and tube building in the polychaete *Mesochaetopterus taylori* Potts. *Can. J. Zool. Rev. Can. Zool.* 73:509-517.

Shirley, T.C. 1990. Ecology of *Priapulus caudatus* Lamarck, 1816 (priapulida) in an Alaskan subarctic ecosystem. *Bull. Mar. Sci.* 47:149-158.

Smith, T.B. 1983. Tentacular ultrastructure and feeding behavior of Neopentadactyla mixta. *J. Exp. Mar. Biol. Ecol.* 61:85-98.

Sokolova, M.N., N.G. Vinogradova, and I.I. Burmistrova. 1995. The 'rain' of dead material as a food source for ultraabyssal macrobenthos in the Orkney Trench. *Okeanologiya* 35:587-591.

Stanley, S.M. 1975. Why clams have the shape they have: an experimental analysis of burrowing. *Paleontology* 1:48.

Appendix 6 continued.

Tamaki, A. 1987. Comparison of resistivity to transport by wave action in several polychaete species on an intertidal sand flat. *Mar. Ecol. Prog. Ser.* 37:181-189.

Turbeville, J.M. and E.E. Ruppert. 1983. Epidermal muscles and peristaltic burrowing in *Carinoma tremaphoros*: correlates of effective burrowing without segmentation. *Zoomorph.* 103:103-120.

Vavelle, J. and M. Grasset. 1990. New data about the make up and the composition of the larval tube of *Pectinaria* (= *Lagis*) *koreni* Malmgren (Annelid, Polychaete). *Cah. Biol. Mar.* 31:333-348.

Wells, G.P. 1959. Worm autobiographies. *Sci. Amer.* 200:132-141.

Yun, S. and T. Kikuchi. 1989. Community structure of macrobenthos in Tomioka Bay, Amakusa. *Publ. Amakusa Mar. Biol. Lab.* 10:79-103.

Appendix 7. Biomass/abundance of numerically dominant taxa for the colonization experiment. Means and 95% confidence limits (CL), p values (p) from ANOVA, pairwise comparisons (Tukey's), R, reference; T, tailings; A, ambient, and minimum detectable differences (mdd) as percent of the reference mean (alpha = 0.05, 1-beta = 0.8). Taxa are listed in order: M. californiensis, G. oculata, P. stennstrupi, P. glabra. Data are from Figure 11, Chapter 2.

Sampling period	Reference		Tailings		Ambient			Pairwise comparisons			
	Mean	95% CL	Mean	95% CL	Mean	95% CL	p	R-T	R-A	T-A	mdd
1	0.0109	0.0085 0.0132	0.0095	0.0079 0.0112	0.0240	0.0210 0.0271	0.000	0.657	0.000	0.000	46.1
1	0.0224	0.0166 0.0282	0.0194	0.0159 0.0231	0.0509	0.0321 0.0700	0.000	0.909	0.001	0.000	107.9
1	0.0715	0.0401 0.1039	0.0591	0.0400 0.0785	0.0360	0.0270 0.0450	0.045	0.646	0.038	0.222	64.0
1	0.0106	0.0074 0.0138	0.0108	0.0071 0.0144	0.0192	0.0149 0.0234	0.001	0.997	0.002	0.003	72.4
2	0.0274	0.0222 0.0327	0.0184	0.0130 0.0238	0.0220	0.0173 0.0268	0.029	0.023	0.230	0.495	38.8
2	0.1822	0.1422 0.2237	0.1646	0.1227 0.2079	0.1787	0.1017 0.2611	0.872	-	-	-	65.4
2	0.0640	0.0512 0.0768	0.0978	0.0561 0.1411	0.0559	0.0425 0.0694	0.041	0.153	1.000	0.052	87.9
2	0.0219	0.0173 0.0265	0.0233	0.0160 0.0307	0.0486	0.0231 0.0747	0.017	0.988	0.028	0.039	146.7

Appendix 7 continued.

Sampling period	Reference		Tailings		Ambient			Pairwise comparisons			
	Mean	95% CL	Mean	95% CL	Mean	95% CL	p	R-T	R-A	T-A	mdd
3	0.0347	0.0294	0.0259	0.0212	0.0130	0.0109	0.000	0.008	0.000	0.000	25.6
		0.0401		0.0306		0.0152					
3	0.2918	0.1722	0.2965	0.2329	0.0536	0.0374	0.000	0.996	0.000	0.000	55.5
		0.4237		0.3633		0.0700					
3	0.0445	0.0338	0.0632	0.0541	0.0302	0.0270	0.000	0.004	0.025	0.000	38.7
		0.0553		0.0723		0.0333					
3	0.0624	0.0485	0.0624	0.0421	0.0262	0.0188	0.000	1.000	0.002	0.002	49.7
		0.0764		0.0831		0.0337					

Appendix 8. Abundance by feeding type for the colonization experiment. Means and 95% confidence limits (CL), p values (p) from ANOVA, pairwise comparisons (Tukey's), R, reference; T, tailings; A, ambient, and minimum detectable differences (mdd) as percent of the reference mean (alpha = 0.05, 1-beta = 0.8). Data are from Figure 12, Chapter 2. Feeding types: ssd, subsurface deposit; sd, surface deposit; sd/s, surface deposit/suspension; sd/c, surface deposit/carnivore; c, carnivore; o, other; u, unclassified.

		Reference		Tailings		Ambient			Pairwise comparisons			
Feeding type	Sampling period	Mean	95% CI	Mean	95% CI	Mean	95% CI	p	R-T	R-A	T-A	mdd
ssd	1	240.95	174.30 318.35	168.28	133.97 206.48	73.72	53.62 96.98	0.000	0.064	0.000	0.001	43.1
sd	1	116.02	97.06 136.66	101.15	77.09 128.45	109.25	87.33 133.62	0.594	-	-	-	38.7
sd/s	1	134.47	97.06 177.94	147.67	127.23 169.62	55.20	35.19 79.64	0.000	0.807	0.000	0.000	42.2
sd/c	1	10.41	7.89 13.26	6.90	5.16 8.87	45.22	32.95 59.40	0.000	0.329	0.000	0.000	150.8
c	1	143.26	110.44 180.34	128.75	110.29 148.63	110.51	83.57 141.19	0.195	-	-	-	39.2
o	1	17.92	13.33 23.17	15.35	11.21 20.11	3.38	1.69 5.54	0.000	0.634	0.000	0.000	45.5
u	1	52.61	29.13 82.91	43.82	23.44 70.44	53.38	36.12 73.96	0.765	-	-	-	93.3
ssd	2	245.27	194.16 302.32	112.26	72.11 161.21	60.33	35.17 92.16	0.000	0.001	0.000	0.061	38.8
sd	2	84.44	67.98 102.67	66.25	51.38 83.00	70.13	53.06 89.55	0.232	-	-	-	42.0
sd/s	2	132.73	103.14 166.05	139.02	104.99 177.81	15.92	10.43 22.53	0.000	0.938	0.000	0.000	42.0
sd/c	2	12.92	9.35 17.03	5.84	2.81 9.85	48.39	37.92 60.13	0.000	0.032	0.000	0.000	115.3
c	2	115.54	98.03 134.48	99.82	84.07 116.91	47.75	39.67 56.57	0.000	0.271	0.000	0.000	27.1
o	2	14.82	9.53 21.22	18.65	14.07 23.85	1.37	0.27 2.96	0.000	0.455	0.000	0.000	57.1
u	2	24.49	13.96 37.87	33.28	23.35 44.94	44.79	23.62 72.56	0.147	-	-	-	146.5

Appendix 8 continued.

Feeding type	Sampling period	Reference Mean	Reference 95% CI	Tailings Mean	Tailings 95% CI	Ambient Mean	Ambient 95% CI	p	Pairwise comparisons R-T	R-A	T-A	mdd
ssd	3	277.55	217.79 344.53	198.84	173.11 226.34	112.85	79.05 152.62	0.000	0.039	0.000	0.004	34.0
sd	3	270.55	218.62 327.99	256.91	198.54 322.77	315.72	271.84 362.88	0.230	-	-	-	40.1
sd/s	3	198.42	138.32 269.30	218.21	167.86 275.15	68.62	46.95 94.37	0.000	0.832	0.000	0.000	50.9
sd/c	3	33.36	23.34 45.13	26.08	18.48 34.95	70.65	63.32 78.38	0.000	0.353	0.000	0.000	57.2
c	3	157.66	121.83 198.08	109.90	94.62 126.32	213.54	176.69 253.87	0.000	0.032	0.031	0.000	40.9
o	3	24.22	15.47 34.89	23.90	17.00 31.93	3.62	2.41 5.03	0.000	1.000	0.000	0.000	59.4
u	3	60.37	38.58 86.97	48.99	31.79 69.83	120.01	85.24 160.68	0.001	0.710	0.008	0.001	93.3

Appendix 9. Abundance by sediment association (Sed. ass.) type for the colonization experiment. Means and 95% confidence limits (CL), p values (p), pairwise comparisons (Tukey's), R, reference; T, tailings; A, ambient, and minimum detectable differences (mdd) as percent of the reference mean (alpha = 0.05, 1-beta = 0.8). Data are from Figure 13, Chapter 2. Sediment association type: burr, burrower; tube, tube builder; surface, surface dweller; uncl, unclassified.

Sed. ass. type	Sampling period	Reference		Tailings		Ambient		p	Pairwise comparisons			mdd
		Mean	95% CI	Mean	95% CI	Mean	95% CI		R-T	R-A	T-A	
burr	1	332.5	236.1 445.2	197.1	146.5 255.2	247.4	204.4 294.5	0.016	0.172	0.012	0.436	47.6
tube	1	229.5	198.2 263.0	119.6	89.5 154.0	238.9	213.5 265.8	0.000	0.905	0.000	0.000	25.9
surface	1	114.4	83.5 150.2	98.4	70.7 130.7	99.4	83.9 116.2	0.587	-	-	-	46.7
uncl	1	38.6	18.0 67.0	37.0	27.3 48.2	31.2	16.2 50.8	0.784	-	-	-	97.1
burr	2	275.6	229.6 325.8	163.2	113.0 222.4	151.8	107.5 203.7	0.002	0.003	0.008	0.921	40.4
tube	2	242.3	206.0 281.5	65.9	50.0 84.0	224.0	179.1 273.9	0.000	0.743	0.000	0.000	29.7
surface	2	103.1	86.7 120.8	39.6	32.2 47.9	92.6	77.3 109.3	0.000	0.522	0.000	0.000	28.7
uncl	2	14.8	7.2 24.9	24.7	16.1 35.2	10.9	6.0 17.1	0.036	0.667	0.179	0.031	108.3
burr	3	414.4	347.0 487.7	360.1	285.7 443.2	320.1	283.0 359.5	0.080	-	-	-	32.7
tube	3	473.1	407.3 543.8	297.4	254.7 343.4	467.0	390.7 550.1	0.000	0.989	0.000	0.000	28.7
surface	3	130.7	95.3 171.7	203.6	166.3 244.6	83.6	68.5 100.2	0.000	0.029	0.006	0.000	47.9
uncl	3	20.4	12.6 30.0	49.6	39.4 61.0	21.6	13.8 31.1	0.000	0.970	0.000	0.001	93.2

Appendix 10. 24 h median effect concentrations, (EC50, LC50) and 95% confidence limits (CL) for gold mill effluent. Data are from Figure 1, Chapter 3.

Species	EC50 immobility	CL	EC50 paralysis	CL	LC50	CL
mysid shrimp	16.5	12.7 - 21.3	30.0	24.9 - 36.2	30.5	25.2 - 36.
northern shrimp	21.9	16.5 - 29.2	44.6	41.9 - 47.5	NE	-
red king crab	30.4	28.7 - 32.2	77.7	72.4 - 83.4	ND	-
Pacific herring	86.1	75.1 - 98.7	86.1	75.1 - 98.7	86.1	75.1 - 98.
sheepshea minnows	NE	-	NE	-	NE	-

ND = not determined.
NE = non-existent.

Appendix 11. Number of mobile, immobile, and paralyzed animals at each concentration of gold mill effluent for each observation. Values are the sum of the replicates. Data are from Chapter 3. Species code: rkc, red king crab; ns, northern shrimp; ph, Pacific herring; ms, mysid shrimp; sm, sheepshead minnows.

			Response		
Species	Exposure duration (h)	Effluent (%)	Mobile	Immobile	Paralyzed
rkc	1	0	34	6	0
rkc	1	4	40	0	0
rkc	1	21	37	3	0
rkc	1	37	11	30	0
rkc	1	54	3	36	1
rkc	1	71	0	16	24
rkc	1	94	0	3	37
rkc	3	0	36	3	1
rkc	3	4	38	2	0
rkc	3	21	40	0	0
rkc	3	37	5	34	2
rkc	3	54	0	23	17
rkc	3	71	0	7	33
rkc	3	94	0	1	39
rkc	6	0	38	2	0
rkc	6	4	40	0	0
rkc	6	21	38	2	0
rkc	6	37	3	38	0
rkc	6	54	1	29	10
rkc	6	71	0	8	32
rkc	6	94	0	1	39
rkc	12	0	40	0	0
rkc	12	4	39	0	0
rkc	12	21	40	0	0
rkc	12	37	2	38	1
rkc	12	54	0	14	26
rkc	12	71	0	6	34
rkc	12	94	0	0	40
rkc	24	0	38	2	0
rkc	24	4	39	1	0
rkc	24	21	40	0	0
rkc	24	37	3	38	0
rkc	24	54	6	25	10
rkc	24	71	0	32	8
rkc	24	94	0	6	34
ns	1	0	18	0	0
ns	1	4	18	0	0
ns	1	21	17	1	0

Appendix 11 continued.

Species	Exposure duration (h)	Effluent (%)	Response		
			Mobile	Immobile	Paralyzed
ns	1	37	1	17	0
ns	1	54	0	18	0
ns	1	71	0	4	14
ns	1	94	0	2	16
ns	3	0	15	3	0
ns	3	4	17	1	0
ns	3	21	16	2	0
ns	3	37	6	12	0
ns	3	54	0	5	13
ns	3	71	0	0	18
ns	3	94	0	1	17
ns	6	0	18	0	0
ns	6	4	17	1	0
ns	6	21	13	5	0
ns	6	37	4	14	0
ns	6	54	0	1	17
ns	6	71	0	1	17
ns	6	94	0	0	18
ns	10	0	16	2	0
ns	10	4	18	0	0
ns	10	21	18	0	0
ns	10	37	10	8	0
ns	10	54	0	1	17
ns	10	71	0	0	18
ns	10	94	0	0	18
ns	24	0	17	1	0
ns	24	4	18	0	0
ns	24	21	11	7	0
ns	24	37	7	10	1
ns	24	54	0	1	17
ns	24	71	0	0	18
ns	24	94	0	0	18
ns	27	0	18	0	0
ns	27	4	18	0	0
ns	27	21	18	0	0
ns	27	37	18	0	0
ns	27	54	18	0	0
ns	27	71	18	0	0
ns	27	94	9	9	0
ns	29.5	0	18	0	0
ns	29.5	4	18	0	0
ns	29.5	21	18	0	0
ns	29.5	37	18	0	0

Appendix 11 continued.

Species	Exposure duration (h)	Effluent (%)	Response		
			Mobile	Immobile	Paralyzed
ns	29.5	54	18	0	0
ns	29.5	71	18	0	0
ns	29.5	94	18	0	0
ph	1	0	32	0	0
ph	1	4	31	0	1
ph	1	21	31	0	1
ph	1	37	30	0	2
ph	1	54	26	0	6
ph	1	71	21	0	11
ph	1	94	20	0	12
ph	3	0	32	0	0
ph	3	4	31	0	1
ph	3	21	29	0	3
ph	3	37	24	0	6
ph	3	54	24	0	8
ph	3	71	29	0	3
ph	3	94	16	0	16
ph	6	0	32	0	0
ph	6	4	31	0	1
ph	6	21	28	0	4
ph	6	37	32	0	0
ph	6	54	28	0	4
ph	6	71	29	0	3
ph	6	94	24	0	8
ph	10	0	32	0	0
ph	10	4	31	0	1
ph	10	21	29	0	3
ph	10	37	31	0	1
ph	10	54	28	0	4
ph	10	71	27	0	5
ph	10	94	24	0	8
ph	24	0	32	0	0
ph	24	4	31	0	1
ph	24	21	30	0	2
ph	24	37	28	0	2
ph	24	54	25	0	7
ph	24	71	22	0	10
ph	24	94	13	0	18
ph	26.5	0	27	0	5
ph	26.5	4	30	0	2
ph	26.5	21	29	0	3
ph	26.5	37	30	0	2
ph	26.5	54	28	0	4

Appendix 11 continued.

Species	Exposure duration (h)	Effluent (%)	Response		
			Mobile	Immobile	Paralyzed
ph	26.5	71	18	0	14
ph	26.5	94	9	0	23
ph	48	0	25	0	7
ph	48	4	23	0	9
ph	48	21	29	0	3
ph	48	37	28	0	4
ph	48	54	20	0	12
ph	48	71	17	0	15
ph	48	94	11	0	21
ms	2	0	20	0	0
ms	2	4	20	0	0
ms	2	21	15	5	0
ms	2	37	0	20	0
ms	2	54	0	4	16
ms	2	71	0	6	14
ms	2	94	0	2	18
ms	4	0	20	0	0
ms	4	4	20	0	0
ms	4	21	11	9	0
ms	4	37	1	19	0
ms	4	54	0	2	18
ms	4	71	0	3	17
ms	4	94	0	1	19
ms	7	0	20	0	0
ms	7	4	20	0	0
ms	7	21	11	9	0
ms	7	37	0	19	1
ms	7	54	0	1	19
ms	7	71	0	0	20
ms	7	94	0	0	20
ms	10	0	20	0	0
ms	10	4	19	0	1
ms	10	21	9	11	0
ms	10	37	0	18	2
ms	10	54	0	2	18
ms	10	71	0	0	20
ms	10	94	0	0	20
ms	24	0	20	0	0
ms	24	4	19	0	0
ms	24	21	11	7	2
ms	24	37	0	8	12
ms	24	54	0	0	20
ms	24	71	0	0	20

Appendix 11 continued.

Species	Exposure duration (h)	Effluent (%)	Response		
			Mobile	Immobile	Paralyzed
ms	24	94	0	0	20
ms	26	0	20	0	0
ms	26	4	18	0	0
ms	26	21	17	0	2
ms	26	37	5	3	12
ms	26	54	0	1	19
ms	26	71	0	0	20
ms	26	94	0	0	20
ms	30	0	10	0	0
ms	30	4	13	0	0
ms	30	21	7	0	2
ms	30	37	2	1	12
ms	30	54	0	0	15
ms	30	71	0	0	15
ms	30	94	0	0	10
sm	1.5	0	18	2	0
sm	1.5	4	17	3	0
sm	1.5	21	15	5	0
sm	1.5	37	14	6	0
sm	1.5	54	15	5	0
sm	1.5	71	13	7	0
sm	1.5	94	14	6	0
sm	3	0	16	4	0
sm	3	4	18	2	0
sm	3	21	15	5	0
sm	3	37	11	9	0
sm	3	54	16	4	0
sm	3	71	16	4	0
sm	3	94	7	13	0
sm	6	0	19	1	0
sm	6	4	11	9	0
sm	6	21	14	6	0
sm	6	37	10	10	0
sm	6	54	16	4	0
sm	6	71	15	5	0
sm	6	94	11	9	0
sm	10	0	20	0	0
sm	10	4	15	5	0
sm	10	21	16	4	0
sm	10	37	12	8	0
sm	10	54	15	5	0
sm	10	71	14	6	0
sm	10	94	16	4	0

Appendix 11 continued.

Species	Exposure duration (h)	Effluent (%)	Response		
			Mobile	Immobile	Paralyzed
sm	24	0	18	2	0
sm	24	4	14	6	0
sm	24	21	15	5	0
sm	24	37	14	6	0
sm	24	54	16	4	0
sm	24	71	16	4	0
sm	24	94	11	9	0

Appendix 12. Number of juvenile mysid shrimp at each exposure duration displaying each of four responses to solutions from experiments that addressed major ion toxicity of gold mill effluent. Values are the sum of the replicates. Data are from Chapter 4. Solution code: eff, effluent; sw, synthetic seawater; nsw, natural seawater; sim, simulated effluent. Solution codes that end in Mg, Ca, or Na indicate adjustment of these ion concentrations. Concn. indicates percent effluent or percent simulated effluent, or salinity (ppt) of sw or nsw. Response code: 1, mobile; 2, immobile but constantly moving; 3, immobile but sporadically moving; 4, paralyzed.

			Response			
Exposure Solution	Exposure duration (h)	Concn.	1	2	3	4
eff	1.5	0	20	0	0	0
eff	1.5	4	20	0	0	0
eff	1.5	21	4	15	1	0
eff	1.5	37	0	0	0	20
eff	1.5	54	0	0	5	15
eff	1.5	71	0	0	6	14
eff	1.5	100	0	1	19	0
eff	3	0	20	0	0	0
eff	3	4	20	0	0	0
eff	3	21	3	16	1	0
eff	3	37	0	0	4	16
eff	3	54	0	0	10	10
eff	3	71	0	0	17	3
eff	3	100	0	7	13	0
eff	6	0	20	0	0	0
eff	6	4	20	0	0	0
eff	6	21	3	15	1	1
eff	6	37	0	0	6	14
eff	6	54	0	0	12	8
eff	6	71	0	0	15	5
eff	6	100	0	1	16	3
eff	10	0	20	0	0	0
eff	10	4	20	0	0	0
eff	10	21	5	13	1	1
eff	10	37	0	0	2	18
eff	10	54	0	0	5	15
eff	10	71	0	0	4	16
eff	10	100	0	0	3	17
eff	24	0	18	0	1	1
eff	24	4	19	0	0	1
eff	24	21	6	8	2	3
eff	24	37	0	0	1	19
eff	24	54	0	0	0	20

Appendix 12 continued.

Exposure Solution	Exposure duration (h)	Concn.	Response 1	2	3	4
eff	24	71	0	0	0	20
eff	24	100	0	0	0	20
sw	1.5	12	20	0	0	0
nsw	1.5	12	20	0	0	0
eff/nsw	1.5	37	0	0	4	16
sim	1.5	37	0	0	3	17
eff	1.5	37	0	0	4	16
sim	1.5	100	0	5	15	0
eff	1.5	100	0	5	15	0
sw	3	12	20	0	0	0
nsw	3	12	20	0	0	0
sim	3	37	0	0	4	16
eff/nsw	3	37	0	0	3	17
eff	3	37	0	0	4	16
sim	3	100	0	8	12	0
eff	3	100	0	12	8	0
sw	6	12	20	0	0	0
nsw	6	12	20	0	0	0
eff	6	37	0	0	5	15
sim	6	37	0	0	6	14
eff/nsw	6	37	0	0	6	14
sim	6	100	0	2	10	8
eff	6	100	0	6	11	3
sw	10	12	20	0	0	0
nsw	10	12	20	0	0	0
sim	10	37	0	1	7	12
eff/nsw	10	37	0	0	3	17
eff	10	37	0	1	6	13
sim	10	100	0	2	4	14
eff	10	100	0	2	5	13
sw	3	8	18	0	0	1
sw	3	22	20	0	0	0
simMgCa	3	37	20	0	0	0
simCa	3	37	15	2	2	1
simMg	3	37	0	0	2	18
effMg	3	100	0	11	9	0
effNa	3	100	0	0	18	2
sim	3	100	0	9	11	0